SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-[(2-PHENYL METHYLIDENE) HYDRAZONO]-1, 3-DIHYDRO -2H-BENZO[g]INDOL-2-ONE DERIVATIVES

Dissertation submitted to

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In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

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Submitted by

REGISTRATION No. 26091794

Under the Guidance of

Mr. M. VIJAYABASKARAN, M.Pharm., Ph.D.

SEPTEMBER - 2011

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

J.K.K. NATTRAJA COLLEGE OF PHARMACY

KOMARAPALAYAM – 638183,

TAMIL NADU.

EVALUATION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-[(2-PHENYL METHYLIDENE) HYDRAZONO]-1, 3-DIHYDRO-2H-BENZO[g]INDOL-2-ONE DERIVATIVES"** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in **Pharmaceutical chemistry**, is a bonafide work carried out by **Mrs. Lakshmi .G [Reg.No.26091794]**, during the academic year 2010-2011, under my guidance and direct supervision in the Department of Pharmaceutical Chemistry, J.K.K. Nattraja College of Pharmacy, Komarapalayam.

Internal Examiner **External Examiner**

CERTIFICATES

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Place: Komarapalayam
Date:

Dr. P. Perumal, M.Pharm., Ph.D., AIC.,
Principal & Head
Department of Pharmaceutical Chemistry,
J.K.K. Nattraja College of Pharmacy,
Komarapalayam- 638183

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Place: Komarapalayam
Date:

Mr. M. VIJAYABASKARAN, M.Pharm. Ph.D.
Assistant Professor,
Department of Pharmaceutical chemistry,
J.K.K. Nattraja College of Pharmacy.
Komarapalayam-638183.

DECLARATION

I hereby declare that the dissertation entitled **"SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-[(2-PHENYL METHYLIDENE) HYDRAZONO]-1, 3-DIHYDRO-2H-BENZO[g]INDOL-2-ONE DERIVATIVES"** has been carried out under the direct supervision of **Mr. M. Vijayabaskaran, M.Pharm. Ph.D.** Assistant Professor, Department of Pharmaceutical Chemistry, J.K.K. Nattraja College of Pharmacy, Komarapalayam, in partial fulfillment of the requirements for the award of degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** during the academic year 2010-2011.

I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma associateship and fellowship or any other similar title.

Place: Komarapalayam
Date:

LAKSHMI .G
Regd. No: 26091794
Department of Pharmaceutical Chemistry,
J.K.K. Nattraja College of Pharmacy,
Komarapalayam- 638183.

Dedicated

To

My beloved family,

guide & my Husband.

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LAKSHMI. G

Reg. No: 26091794

CONTENTS

CONTENTS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 3

AIM AND PLAN OF WORK

CHAPTER 4

EXPERIMENTAL WORK

CHAPTER 5

BIOLOGICAL SCREENING

CHAPTER 6

RESULTS AND DISCUSSION

CHAPTER 7

CONCLUSION

CHAPTER 8

BIBLIOGRA

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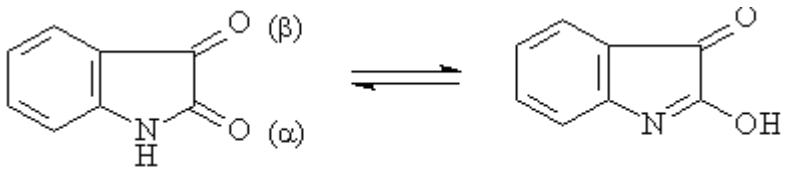
1. INTRODUCTION

1.1 Chemistry of Isatin

Isatin (1H-indole- 2, 3-dione) (I) was first discovered by Erdmann[1] and Laurent[2] in 1841, independently as a product from oxidation of indigo by nitric and chromic acids.



(I) (II)

(Isatin) **(Dioxindole)**

It is a unique molecule possessing both amide and keto carbonyl groups. A part from this, it has an active hydrogen atom attached to nitrogen (or oxygen) and an aromatic ring which was substituted at 5- and 7-positions. It exists in a tautomeric form (II) and these functional characteristics play an important role in governing the various reactions of the molecule.

The C-3 carbonyl group of isatin is strongly electrophilic. As a result, isatins are readily involved in condensation and addition reactions with carbanion type nucleophiles into 3-substituted oxindoles[3]. In general, there are three possibilities during condensation reactions.

i. Both the , α-carbonyl groups, having varying in reactivity are involved,

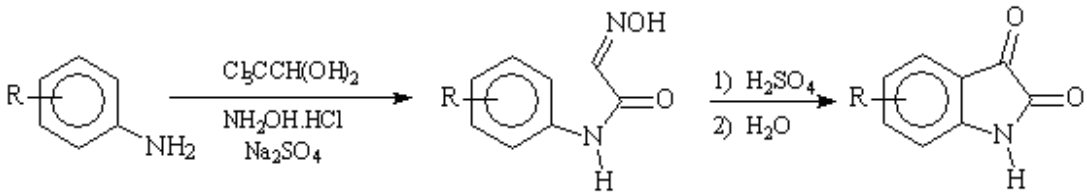
ii. Ring cleavage takes place and

iii. Ring expansion occurs

A general observation reveals that the nature of final product always depends on the experimental conditions and substituent on nitrogen atom which may affect the electron density at β and α carbonyl carbon atoms respectively[4].

The Sandmeyer methodology

This method is applied mostly well to anilines with electron-withdrawing Substituents, such as 2-fluoroaniline[5] **(1)**.



(Substituted aniline) **(Isonitrosoacetanilide)** **(Substituted isatin)**

(1)

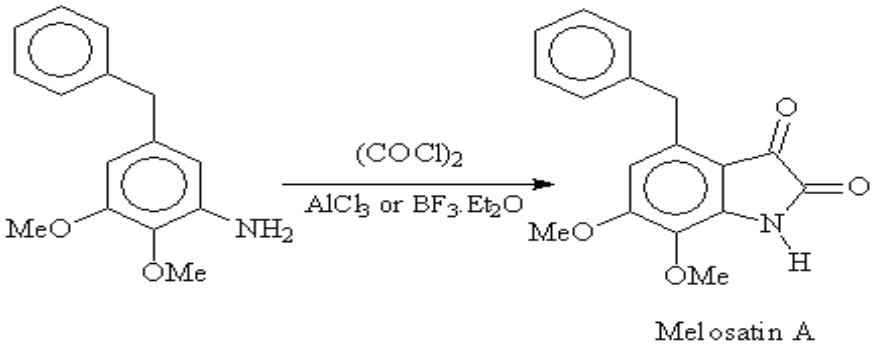
Scheme-1

In addition to the use of H_2SO_4 for the cyclization step, isonitrosoacetanilide is heated in $BF_3.Et_2O$ at 90°C. After cooling the reaction mixture, addition of water allows isolation of the respective isatins. This methodology has been proved to be particularly effective for the preparation of benzo-oxygenated isatin derivatives[6, 7].

The Stolle procedure

The most important alternative to Sandmeyer's procedure is the method of Stolle. In this method anilines react with oxalyl chloride to form an intermediate chloro-oxalylanilide which can be cyclized in the presence of a Lewis acid, usually aluminium chloride or $BF_3.Et_2O$, although $TiCl_4$ has also been used to give the corresponding isatin. This method has been used for the synthesis of 1-aryl[8] and polycyclic isatins derived from phenoxazine, phenothiazine and dibenzoazepine[9] as well as indoline[10]. In the case of dimethoxyanilines, spontaneous cyclization to

yield dimethoxyisatins in the absence of a Lewis acid has been observed, as exemplified in the synthesis of melosatin A **(2)**, albeit in very low yield (Scheme-2).



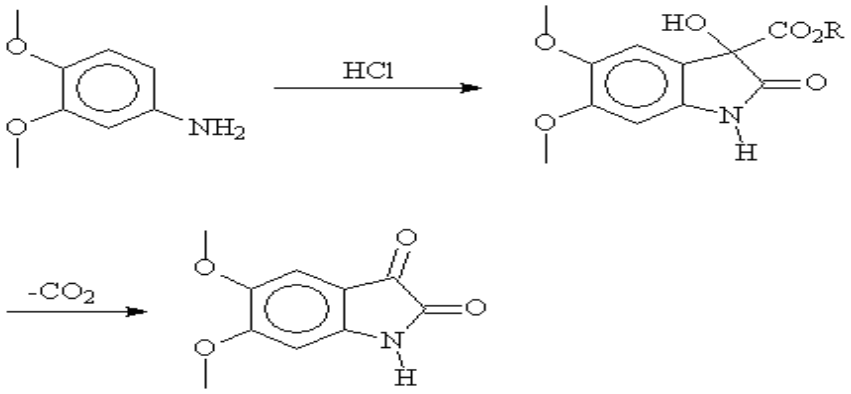
Melosatin A

(2, 3-Dimethoxy (5-methyl benzyl) aniline)

(2)
Scheme-2

The Martinet Isatin synthesis

The Martinet procedure for the synthesis of indole-2,3-diones involves the reaction of an amino aromatic compound and either an oxomalonate ester or its hydrate in the presence of an acid to yield a 3-(3-hydroxy-2-oxindole)carboxylic acid derivative which after oxidative decarboxylation yields the respective isatin **(3)**. This method was applied successfully for the synthesis of 5, 6-dimethoxy isatin from 4-aminoveratrole whereas the use of 2, 4-dimethoxyaniline was less successful[11].



(5, 6-Dimethoxy isatin)

(3)

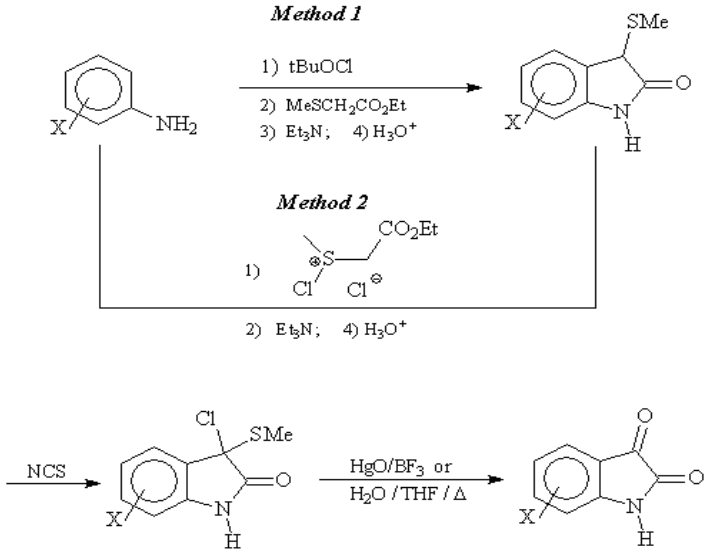
Scheme-3

The Martinet procedure is readily applied to naphthylamines, thus yielding benzoisatin derivatives[12].

The Gassman procedure

A fundamentally different and general procedure developed by Gassman is another option for the synthesis of isatins[13]. This methodology consists in the formation and subsequent oxidation of an intermediate 3-methylthio-2-oxindole to give the corresponding substituted isatins (**4**) in 40-81% yield.

Two complementary methods for the synthesis of the 3-methylthio-2-oxindoles were developed, and the methodology of choice is dependent upon the electronic effect of substituents bonded to the aromatic ring. When electron-withdrawing groups are present, the oxindole derivative can be synthesized via *N*-chloroaniline intermediate, which further reacts with a methyl thioacetate ester to furnish an azasulfonium salt (Method 1, Scheme 4). In the case of electron-donating groups that destabilize the *N*-chloro intermediate, and thus give diminished yields of the azasulfonium salt, a second method of generation of this salt, by reaction of the chlorosulfonium salt with appropriate aniline, gives better yield of the 3-methylthio-2-oxindoles.

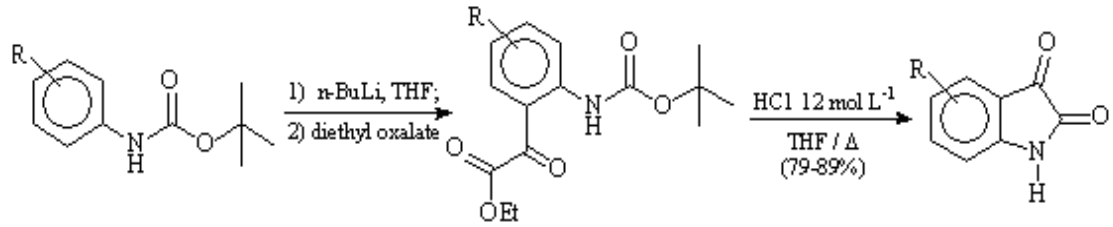


(4) (Substituted isatin)

Scheme-4

Metalation of anilide derivatives

A more recent method for the synthesis of isatins is based upon the directed *ortho*-metalation (D*o*M) of *N*-pivaloyl- and *N*-(*t*-butoxycarbonyl)-anilines. The corresponding dianions are treated with diethyl oxalate and the isatins are obtained after deprotection and cyclisation of the intermediate β-ketoesters. This method has the advantage of being regioselective for the synthesis of 4-substituted isatins **(5)** from *meta*-substituted anilines where the substituent is a metalation directing group (e.g., OMe) [14](Scheme-5).

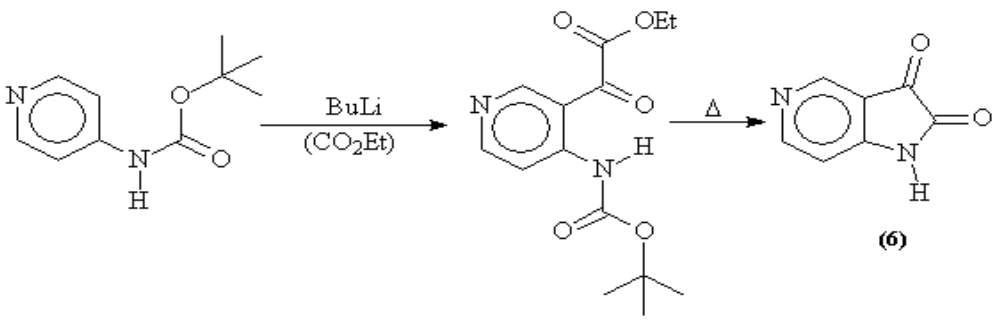


(5)

(N-(*t*-butoxycarbonyl)-aniline) **(4-Substituted isatin)**

Scheme-5

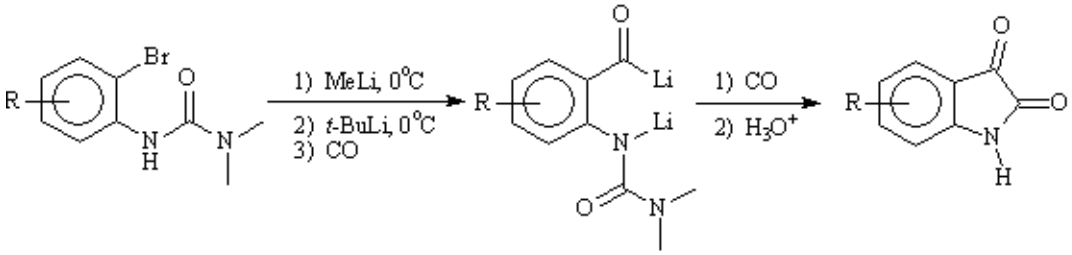
The synthesis of 5-azaisatin was realized by *ortho*-lithiation of the 4-aminopyridine *t*-butylcarbamate followed by reaction with an excess of diethyl oxalate. Heating the glyoxylic ester under vacuum gave 5-azaisatin[15] **(6)** (Scheme 6).



(4-Aminopyridine *t*-butylcarbamate) **(5-Azaisatin)**

Scheme-6

Recently, a metal-halogen exchange method was described for the synthesis of isatins by lithiation of *ortho*-bromophenylureas, carbonization and subsequent intermolecular cyclisation to give the desired products **(7)** in 71-79% yield[16] (Scheme-7).



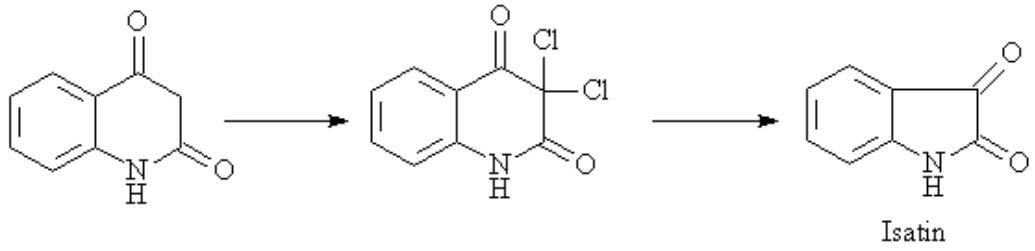
(*O*-Bromo phenylurea) **(Substituted isatin)**

Scheme-7 **(7)**

Other methods

A) Ring contraction method[17]

A number of 2, 4-dihydroxy quinolones, after the introduction of a 3-hydroxyimino, 3-nitroso, 3-hydroxy-3-amino or 3, 3-dihalo groups gives isatins **(8)** with sulphuric acid or sodium hydroxide (Scheme-8).

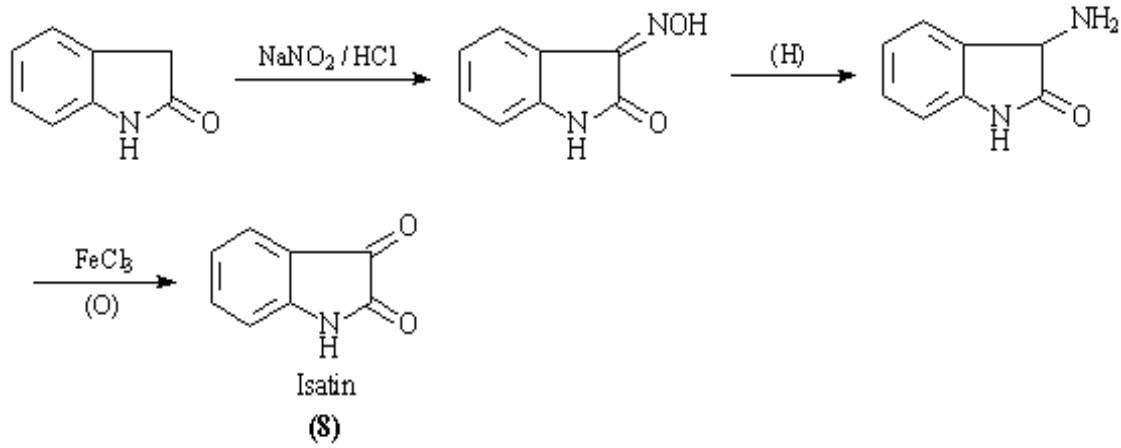


(2, 4-Dihydroxy quinolone) **(3, 3-Dichloro (2, 4-dihydroxy) quinolone)** **(8)**

Scheme-8

B) From indole derivatives[18]

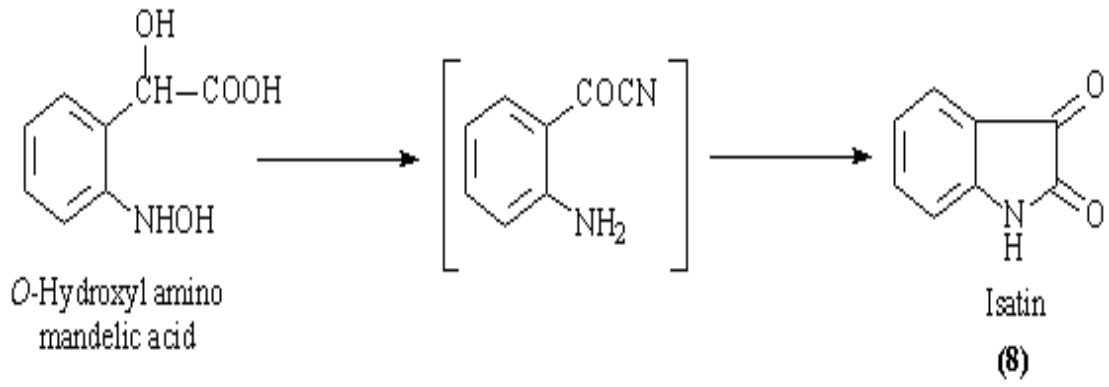
A number of oxindoles have been treated with nitrous acid to give isatin-3-oximes. Reduction of the oximes to 3-amino oxindoles followed by ferric chloride oxidation gives Isatins (Scheme-9).



Scheme-9

C) Heller's method[19]

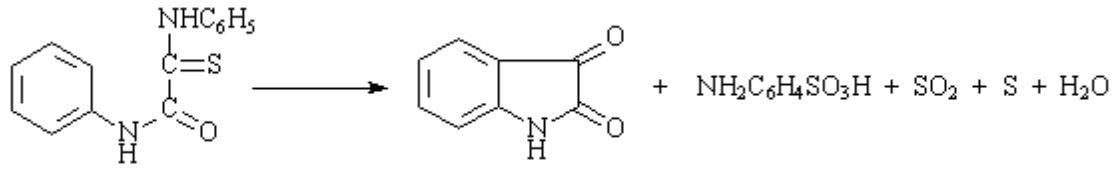
In this method isatin is obtained by heating o-hydroxyl amino mandelic acid with hydrochloric acid (Scheme-10).



Scheme-10

D) Reissert's method [20]

A synthesis developed by Reissert depends on heating thio-oxanilide with conc. sulphuric acid (Scheme-11).

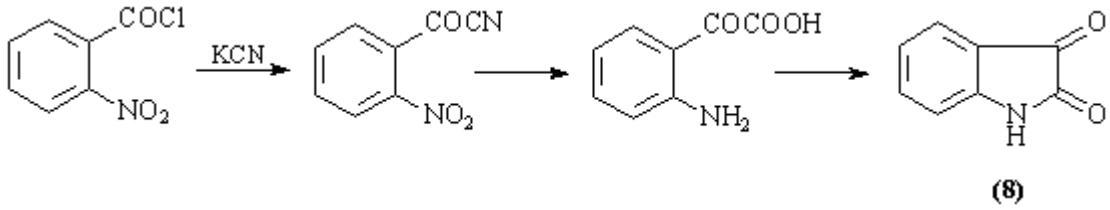


(N-Hexyl thio-oxanilide) **(Isatin)**

Scheme-11

E) Claisen-Shadwell synthesis[21]

O-Nitro benzoyl cyanide prepared from *o*-nitro benzoyl chloride and potassium cyanide can be hydrolyzed and then reduced to the salt of *o*-amino phenyl glycoxylic acid with ferrous hydroxide. Acidification of the solution of glyoxylate affords Isatin **(8)** (Scheme-12).



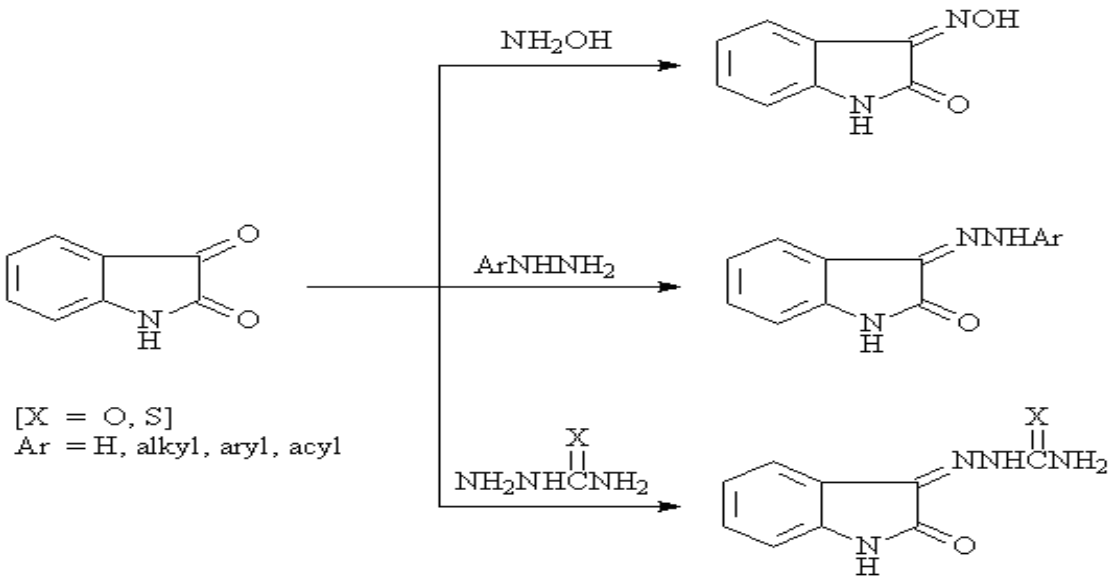
(8)

(*O*-nitro benzoyl chloride) **(*O*-amino phenyl glyoxalicacid)** **(Isatin)**

Scheme-12

1.2 Reactions of Isatin

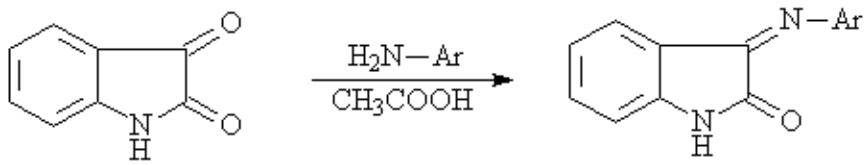
The reactive carbonyl group at position-3 undergoes typical reactions with the ketonic reagents such as hydroxylamine[22], phenyl hydrazine[23] and semicarbazide[24]. The carbonyl group at position-2 is less active and has less ketonic character compared with carbonyl group at C_3 (Scheme-13).



Scheme-13

Schiff bases

Isatin reacts with a variety of aromatic amines[25] in the presence of glacial acetic acid to yield Schiff bases **(9)** (Scheme-14).

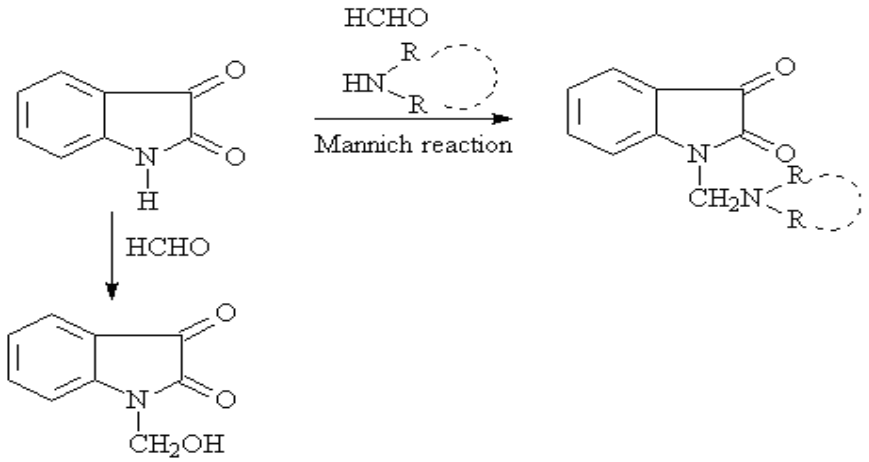


n) Isatin-3-imine **(9)**

Scheme-14

Mannich reaction[26]

Isatin reacts with formaldehyde and a variety of amines in the Mannich reaction to give their respective Mannich bases, in the absence of an amine, isatin and substituted isatin with formaldehyde give hydroxymethyl isatins (Scheme-15).

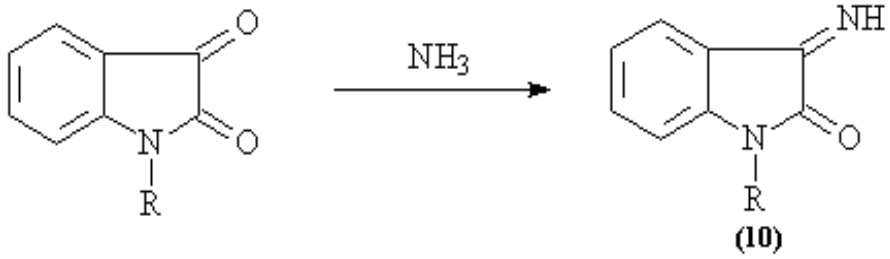


(Hydroxy methyl isatin)

Scheme-15

Aliphatic primary amines

Primary aliphatic amines[27] are also reported to give imines with isatin. Isatin and N-methyl isatin can be reacted with ammonia to give imines' **(10)** (Scheme-16).



(10)

(N-Substituted isatin) **(N-Substituted imine)**

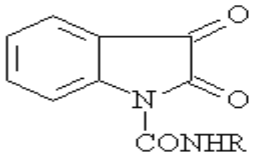
Scheme-16

N-Substituted Isatins

Acylation and Alkylation

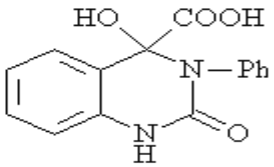
Isatin sodium or potassium or silver salts react with dimethyl or diethyl sulfate, a variety of alkyl halides, and anhydride[28] to give N-alkyl and N-acyl isatins.

Isatin and triethylamine with phenyl and methyl isocyanate has reported to give 1-carboxamidoisatin[29] **(11)**. In alkali, however isatin and phenyl isocyanate or phenyl isothiocyanate to give the quinazolines[30] **(12)**.



(11)

(1-Carboxamidoisatin)



(12)

(Substituted quinazoline)

Other reactions of Isatins

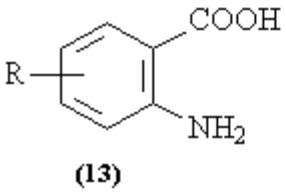
a) Electrophilic substitution and related reactions

Reagents such as N-bromosuccinimide, N-chlorosuccinimide, N-bromo caprolactam, sulfuryl chloride, bromine, t-butyl hypochlorite and I or Cl have been used to prepare 5-haloisatins. Chlorination (halogenations) of 6 and 4-chloroisatin, alkylation leads to halogenation at 5 position[31-34]. If the 5-position is occupied halogenation takes place in the 7-position.

Nitration of isatin, 7-methyl isatin, 4,7-dimethyl isatin and 1-ethyl isatin leads to the introduction of the nitro group into the 5-position. Nitration of 5-methyl isatin gives 5-methyl-7-nitro isatin.

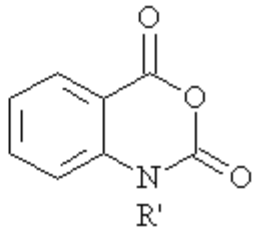
b) Oxidation

Oxidation reaction of isatin yields anthranilic acid with alkaline hydrogen peroxide. The oxidation has been applied to alkyl, halo, alkoxy, trifluro methyl and nitro isatins. Oxidation with chromium trioxide in acetic acid, chromium trioxide in acetic anhydride – acetic acid[35], 3-chloro per benzoic acid **(13)** in methylene chloride[36] or benzene give rise to isatoic anhydride **(14).**



(13)

(3-Substituted perbenzoic acid)

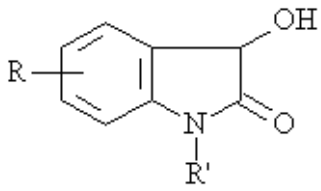


(14)

(Isatoic anhydride)

c) Reduction

Isatin derivatives when reduced with sodium hydro sulfite, zinc-copper leads to the corresponding oxindole **(15)**.



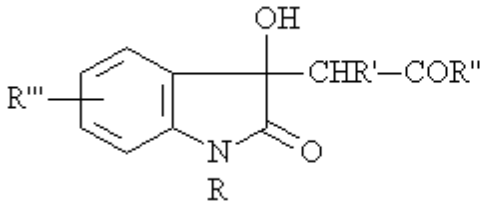
(Substituted oxindole)

(15)

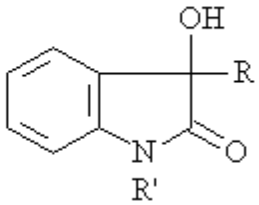
Lithium Aluminium hydride reduction of isatin gives mixture of 3- hydroxy indole, indole, indigo and indirubin. However, $lialh_4$ is used to convert, 4,5,6-trimethoxy isatin, 5-bromo isatin, 4,6-dimethoxy isatin, 1-ethyl and 1-methyl isatin to the corresponding indoles. Catalytic hydrogenation of isatin gives oxindoles, whereas electrolytic reduction using a lead electrode gives dihydroxy indole[37].

d) Condensation involving carbon at C-3

Isatin on condensation with a wide variety of ketones yields 3-hydroxy-3-substituted oxindoles[38] **(16, 17)**.



(16)

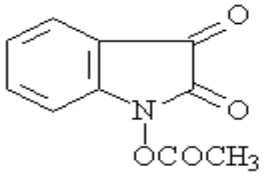


(17)

Isatin and its derivatives have reacted with both aryl and alkyl Grignard reagents to give 3-alkyl (or aryl) dioxindoles.

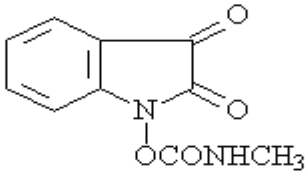
e) Reaction of N-substituents

Reaction of N-hydroxy isatin with acetic anhydride gives compound[39] **(18)** whereas methyl isocyanate in phenyl diazo methane gives compound[40] **(19)**. Isatin Mannich reaction products have been reacted with active hydrogen compounds to yield their respective Mannich bases[41] **(20)**.



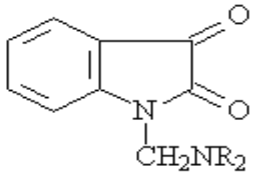
(N-Acetyl isatin)

(18)



(N-(N-Methyl amido) isatin)

(19)



(N-(N-Substituted methyl) isatin)

(20)

2. REVIEW OF LITERATURE

Isatin and several of their derivatives have been generally associated with various biological and pharmacological properties. The synthesis of a large number of isatin derivatives have been described to obtain biologically potent compounds. Many such compounds have been found to be promising. A few even have clinical application also.

This prominence aroused interest to several chemists and medicinal chemists to prepare day to day newer and newer potential isatin derivatives by molecular conjunction approach and evaluating them for possible pharmacological actions.

Since there have been numerous reports, it is highly impossible to cover all such reports in a single review. Hence, it is chosen to present some interesting reports of the recent two decades on the topic, mainly to indicate the recent trends in the progress of research on isatin derivatives. It is needless to quote that it is not at all an easy task to cover even these two decades, meticulously. However, efforts have been made to cover very broadly but briefly.

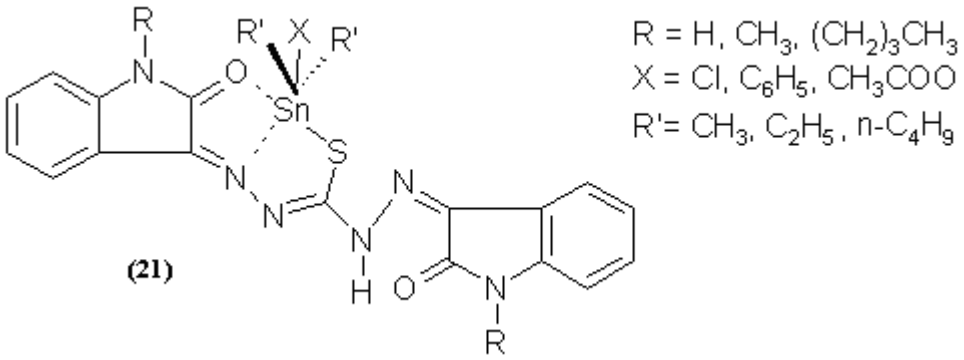
The present survey aims to synthesize of some new isatin derivatives of specific biological and pharmacological activity and looking for such activity (or) activities by their evaluation experimentally.

Since there have been numerous reports, some of the interesting reports are presented here according to their biological / pharmacological activity for orderly presentation.

ISATINS AS ANTIMICROBIAL AGENTS

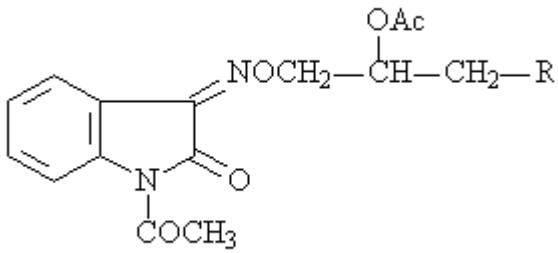
Isatin itself is inhibitory to the growth of Tubercle bacillus. Isatin-β-thiosemicarbazone was described as antibacterial by various workers. This compound was found to be effective against tubercle bacillus to some extent.

M. Carcelli et *al.,* [42] reported the properties of organotin (IV) complexes with isatin and N-alkalization bisthiocarbohydrazones **(21)** against antimicrobial and mutagenic property.



(21)

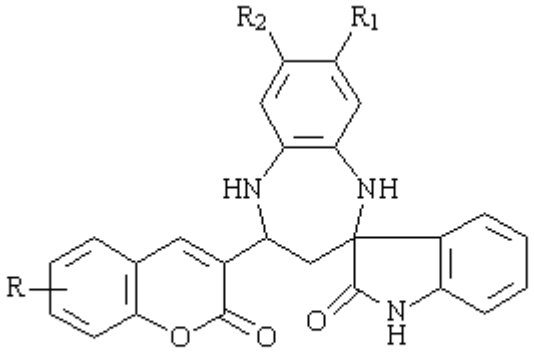
Padhy *et al.,* [43] reported the synthesis of 1-acetyl-3-(2-acetoxy-3-substituted propyloximine) indol-2-(3H)-ones (**22**) and evaluated for antimicrobial activity against *B. subtilis, E. coli* and *C. albicans*. Among them, the compound with 2-acetoxy-3-substituted propyloximino chain at 3-position of indole-2, 3-dione exhibited high activity with MIC of 0.35 g/ml to 12.5 µg/ml against *B. subtilis*, 0.16 – 3.12 µg/ml against *E. coli* and 6.2 – 100 µg/ml against *C. albicans*.



(22)

R – Piperidino; pyrrolidino; dicyclohexylamino; diphenylamino; methylphenylamino

Ravi raj A. Kusanur *et al.,* [44] reported the synthesis of 4'-(coumarin-3-yl) spiro [3H-indol-3,2'-1,5-benzodiazepine]-2(1H)-one **(23)** and evaluated for *in vitro* antibacterial activity.

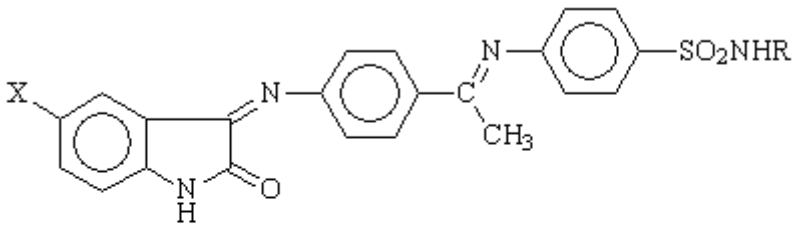


R = H, 6-CH_3, 8-OCH_3, 5,6-benzo, 6-Cl, 6-Br; $R_1 = R_2$ = H, CH_3

(23)

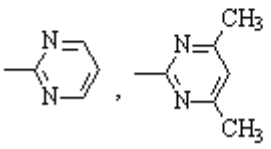
Among all the compounds, compound with R = 8-OCH_3, $R^1 = R^2 = CH_3$ showed 88.83% of inhibition against *B. subtilis* and 77.77% of inhibition against *E. coli* as compared to the standard and other compounds were moderately active.

Gupta *et al.,* [45] reported the synthesis of 1,3-dihydro-5-substituted-3-[[4-[1-(p-sulphamylphenylimino)ethyl)phenyl]imino]-2H-indol-2-ones **(24)** and screened them for *in vitro* antibacterial activity. Compounds with X = H, R = diazino; X = Cl, R = H; X = Cl, R = -C (=NH) NH2; X = Cl, R = diazino and X = Cl, R = dimidino showed highest activity against *B. subtilis* and *S. aureus*.



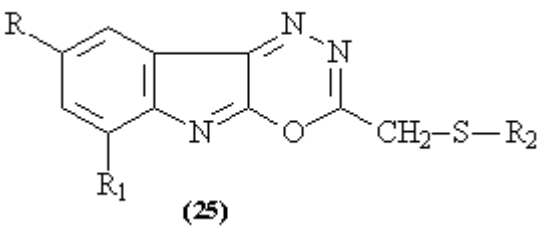
X = -H, Cl, -CH_3

R = H, -C(=NH)NH_2,



(24)

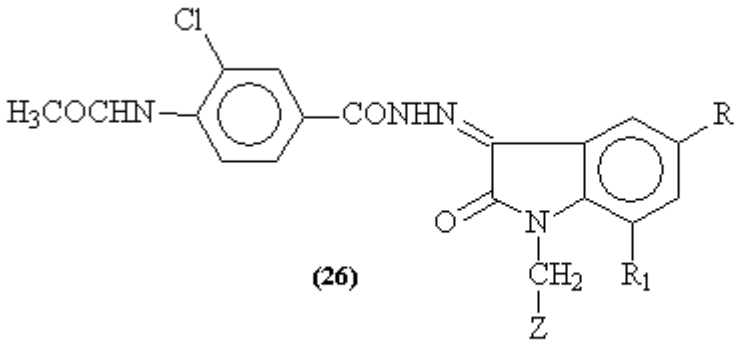
Ajitha *et al.,* [46] reported the synthesis of new 2-substituted-[1, 3, 4]-oxadiazino-[6, 5-b]-indoles **(25).** The compounds were evaluated for *in vitro* antimicrobial activity. Compounds (R = Cl, R_1 = H, R_2 = benzimidazolyl), (R = Br, R_1 = H, R_2 = benzimidazolyl) showed highest antimicrobial activity which was comparable with that of Ampicillin.



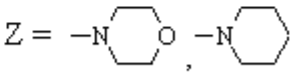
(25)

R = H, CH_3, Cl, Br
R_1 = H, CH_3
R_2 = benzimidazolyl,
4,5-diphenylimidazolyl,
5-phenyl-1,3,4-oxadiazolyl,
5-phenyl-1,3,4-thiadiazolyl.

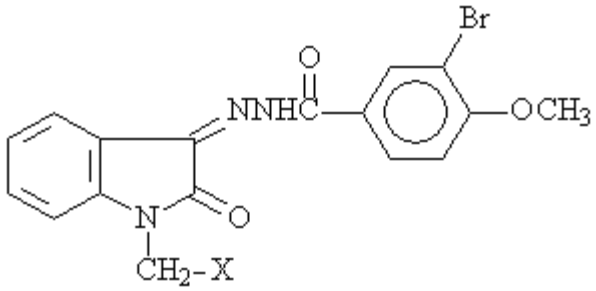
Varma and Vinita Bajpai [47]prepared a new series of 1-heteryl aminomethyl-3-(4'-acetylamino-3'-chlorobenzoylhydrazono)-2-indolinones **(26)** as potential antimycotics. Amongst the compounds tested, the compounds in which (Z = morpholino, piperidino, R = H, Br) respectively displayed significant activity against *T. Mentha* grophytes (MIC – 50 µg/ml)



(26)

R = H, Cl, Br, CH_3,
R_1 = H, Br
Z = 

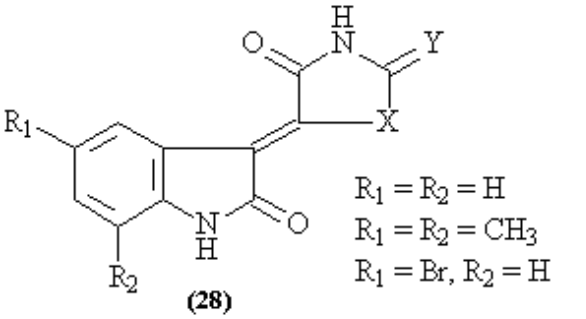
Havaldar and Mishra [48]prepared 1-(substituted amino methyl)-3-(3'-bromo-4'-methoxybenzoylhydrazone) indolin-2-ones **(27)** and screened for *in vitro* antibacterial and antifungal activities. Compound with X = morpholino, piperidino showed highest activity against *S. aureus* and *C. albicans.* .



X = anilino, 4-methoxyanilino,
N-methylanilino, morpholino, piperidi

(27)

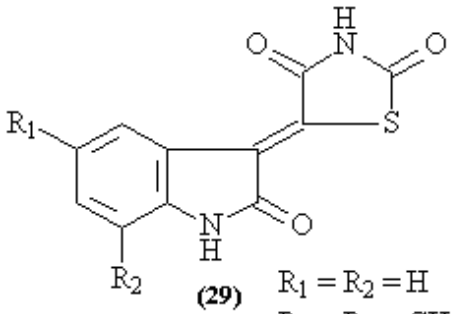
Pardasani *et al.,* [49]prepared some fused and Spiro imidazolidine derivatives **(28, 29)** and subjected for antimicrobial activity. The compounds showed significant inhibitory activity against two bacteria.



(28)

$R_1 = R_2 = H$
$R_1 = R_2 = CH_3$
$R_1 = Br, R_2 = H$

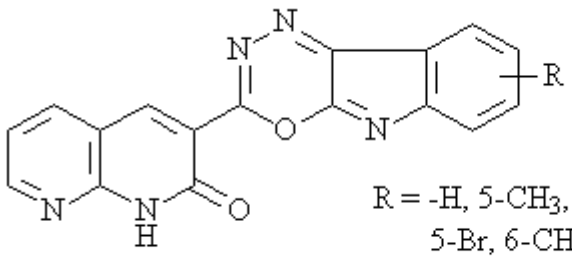
$X = NH, Y = O$
$X = NCH_3, Y = O$



(29)

$R_1 = R_2 = H$
$R_1 = R_2 = CH_3$
$R_1 = Br, R_2 = H$

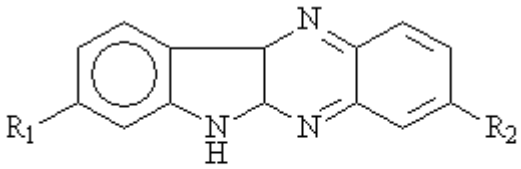
Mogilaiah *et al.,* [50] reported the synthesis and antibacterial activity of some new [1, 3, 4] oxadiazino [6, 5-b]indoles **(30)**, Compounds with R = 6-Cl & *p*-chlorophenyl showed significant inhibitory activity against *E. Coli* and *B. Subtilis.*



(30)

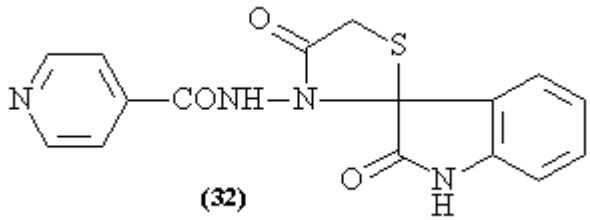
R = -H, 5-CH_3, 7-CH_3, 5-OCH_3, 5-Cl
5-Br, 6-CH_3, 8-CH_3, 6-OCH_3, 6-Cl

Anees A. Siddiqui *et al.,* [51] reported the synthesis of 2', 3' [b]-indolyl-1, 4-quinoxaline derivatives **(31)** and evaluated them for antibacterial activity. The compounds exhibited very mild antibacterial activity.



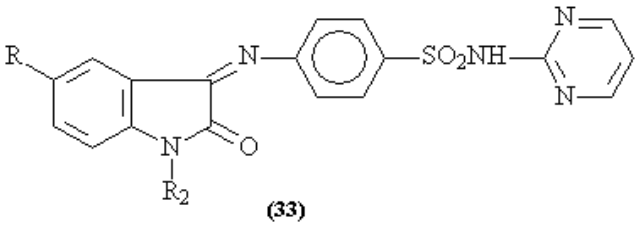
(31) $R_1 = H, Br, NO_2$; $R_2 = H, NO_2$

Alagarsamy *et al.,* [52] prepared 3-(pyridyl-4-carbonylamino) Spiro (3H-indol-3, 2-thiazolidine)-2, 4'-(1H) Dione **(32)** and evaluated for antimicrobial activity. The compound showed equipotent activity with that of standard drug employed.



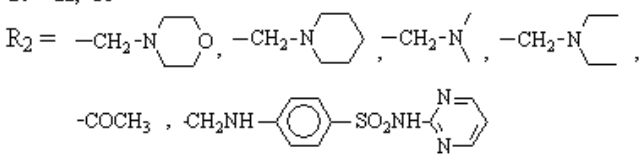
(32)

Pandeya *et al.,* [53] prepared N-Mannich bases of 3-(4-sulphadiazinyl)-isatins **(33)** and screened them for *in vitro* antimicrobial activity.



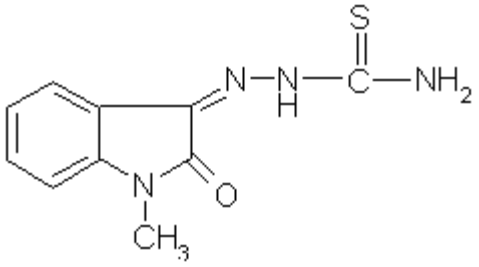
(33)

R = H, Cl

R_2 = 

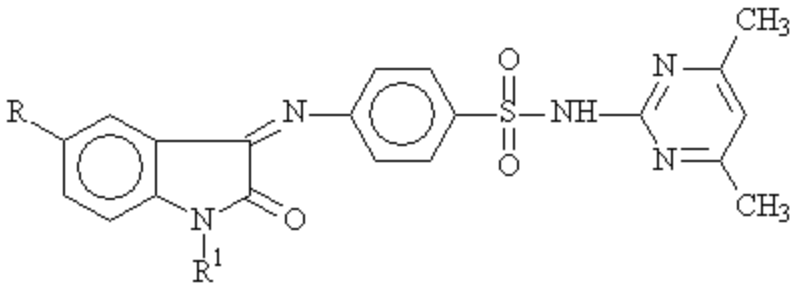
ISATIN AS ANTIVIRAL AGENTS

Debra C.Quenelle *et al.,* [54] reported the synthesis of isatin β-thiosemicarbazone and N-methyl-isatin-β-thiosemicarbazone **(34)** for in vitro and in vivo evaluation against Cowpox virus infections.



(34)

Selvam et al., [55] reported 4-[(1, 2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N-(4, 6-dimethyl-2-pyrimidinyl)-benzene sulphonamide and its derivatives. They were synthesized by the reaction of isatin and its derivatives with sulphadimidine. The anti-HIV activity of the compounds was tested against replication of HIV-1 (IIIB) and HIV-2 (ROD) strains in acutely infected MT-4 cells and the activity was compared with the standard Azidothymidine.

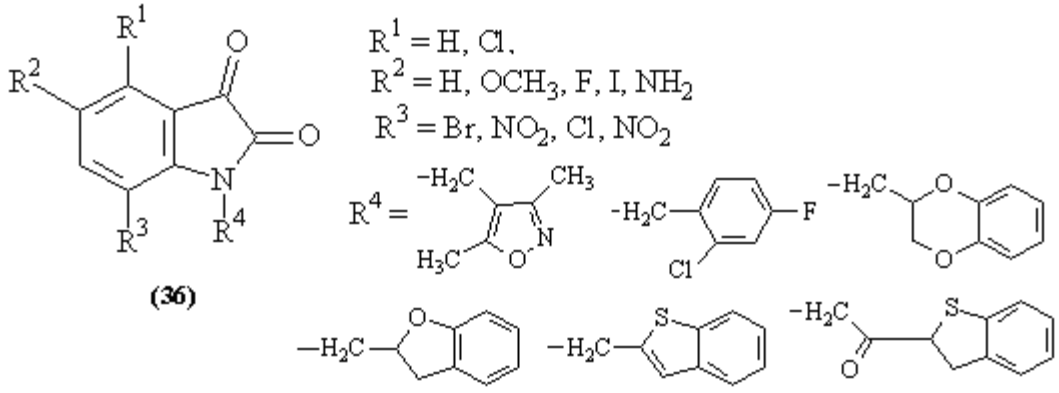


(35)

R = H, Cl, Br, CH_3; R^1 = H, $COCH_3$

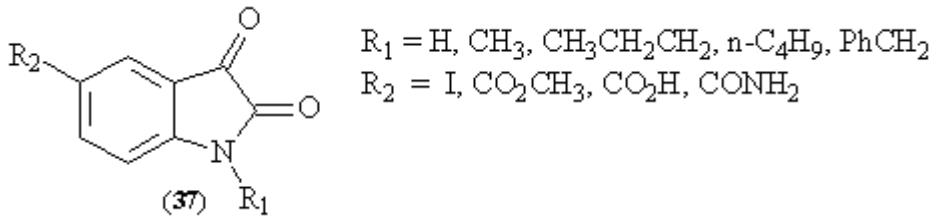
Among the compounds tested, 4-[(1, 2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N-(4, 6-dimethyl-2-pyrimidinyl)-benzene sulphonamide (**35**) and its N-acetyl derivatives were the most active compounds.

Shin-Hun Juang *et al.,* [56] were synthesized N-substituted isatin derivatives **(36)** from the reaction of isatin and various bromides via two steps. Bioactivity assay results (*in vitro* test) demonstrated that some of these compounds are potent and selective inhibitors against SARS corona virus 3CL protease with IC_{50} values ranging from 0.95 to 17.50 μM



(36)

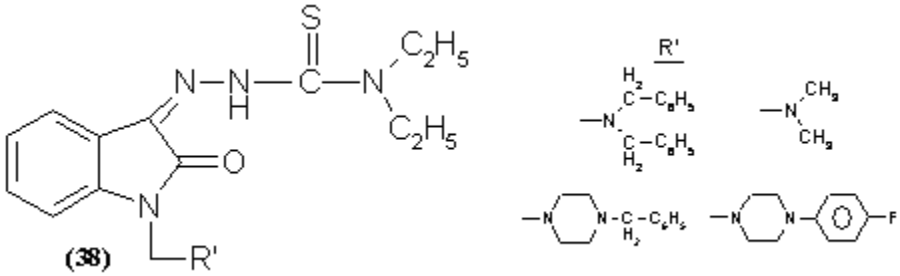
Luhua Lai and Lu Zhou [57], reported, a series of N-substituted isatin derivatives (**37**) were synthesized and tested against SARS CoV 3C-like protease using a colorimetric assay and confirmed by HPLC. The compounds were shown to be noncovalent reversible inhibitors of SARS CoV 3C-like protease. The C-5 position was found to favour a carboxamide group and N-1 position to favour large hydrophoretic substituents.



(37)

The lowest IC_{50} value (0.37 μM) was observed with compound if and it was selective for SARS CoV 3C-like protease over other protease.

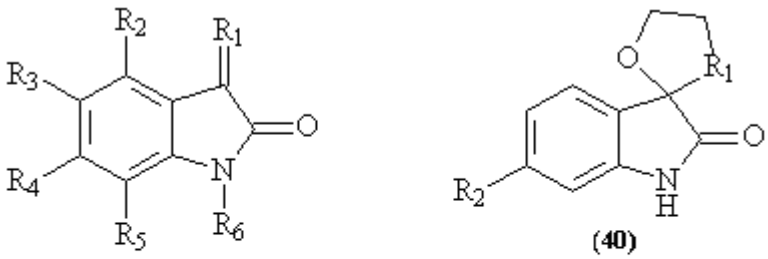
Dharmarajan Sriram *et al.,* [58] reported a series of isatin β-thiosemicarbazone derivatives **(38)** was synthesized and evaluated for their anti-HIV activity in HTLV-III_B strain in CEM cell line.



(38)

ISATINS AS ANTICANCER AGENTS

Kara L-vine *et al.,* [59] reported the *in vitro* cytotoxicity evaluation of some substituted isatin derivatives **(39, 40).**



(39) **(40)**

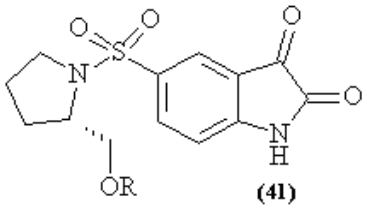
$R_1 = O, N\text{-}C_6H_5, N\text{-}NHC_6H_5$

$R_2 = H, Br;\quad R_3 = H, Br, F, I, NO_2, OCH_3$

$R_4 = H, Br,\quad R_5 = H, Br, NO_2,\quad R_6 = CH_3$

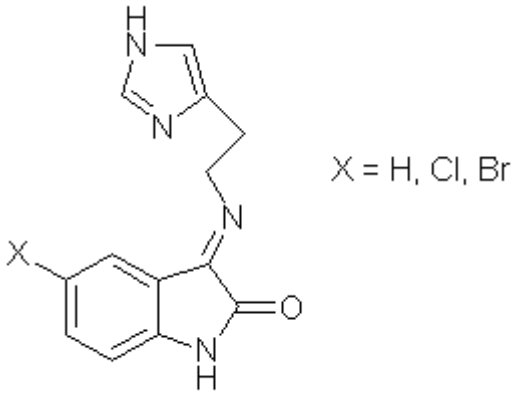
Twenty three compounds were synthesized and tested four were selected for further screening against a panel of five human cancer cell lines. These compounds, in general, showed greater selectivity reward leukemia and lymphoma cells over breast, prostate and colorectal carcinoma cells.

Klaus Kopka *et al*., [60] reported 5-pyrrolidinyl sulfonyl **(41)** isatins as a potential tool for the molecular imaging of carpases in apoptosis.



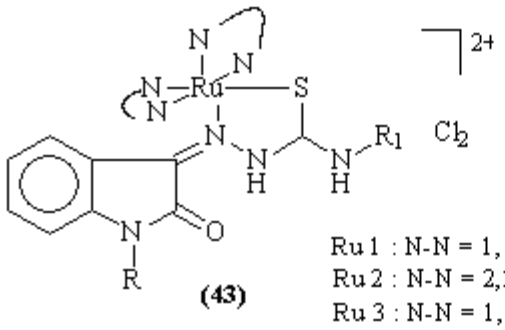
(41)

Ashraf H. Abadi *et al*., [61] reported synthesis of 3-substituted-2-oxoindole **(42)** analogues evaluated anti cancer activity.



(42)

Upal Kanti Mazumder *et al.,* [62] reported the synthesis, anticancer and antibacterial activities of some novel mononuclear Ru (II) complexes **(43).**

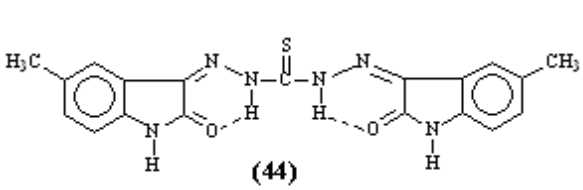


(43)

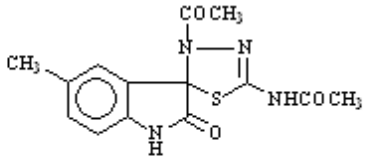
Ru 1 : N-N = 1, 10-Phenanthroline, R = CH_3, R_1 = H
Ru 2 : N-N = 2,2'-bipyridine; R = CH_3, R_1 = H
Ru 3 : N-N = 1,10-Phenanthroline, R = H, R_1 = 4-Cl-phenyl
Ru 4 : N-N = 2,2'-bipyridine, R = H, R_1 = 4-Cl-Phenyl

All the complexes were tested for their anticancer activity against EAC bearing mice. RU1-RU4 was found to increase the life span of the tumor hosts by 66-43% and was the most active in the series of synthesized complexes. RU1-RU4 was also found to bring the altered haemoglobin and RBC values of the EAC bearing mice to near normal values.

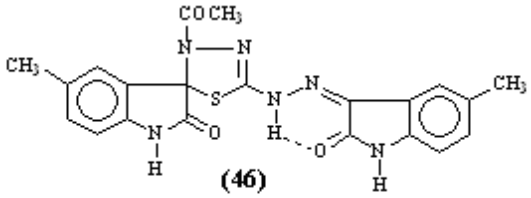
Rafiqul Islam *et al.,* [63] reported the synthesis of 5-spiro (5'-methylisatin)-4-acetyl-2-(acetyl amino)-μ^2-1,3,4-thiadiazoline and 5-spiro (5'-methylisatin-3'-hydrazino)-Δ^2-1, 3, 4-thiadiazoline **(44-47).**



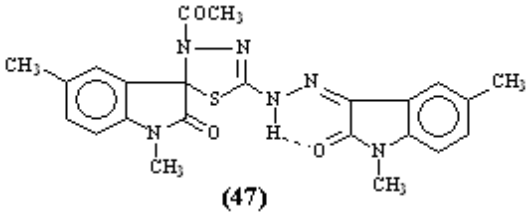
(44)



(45)



(46)

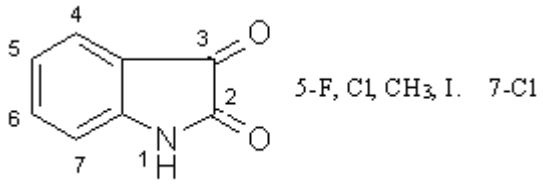


(47)

The anticancer activity of the synthesized compounds was carried out by Brine Shrimp Lethality test and *in vitro* anticancer cell lines assay against various cancer cells.

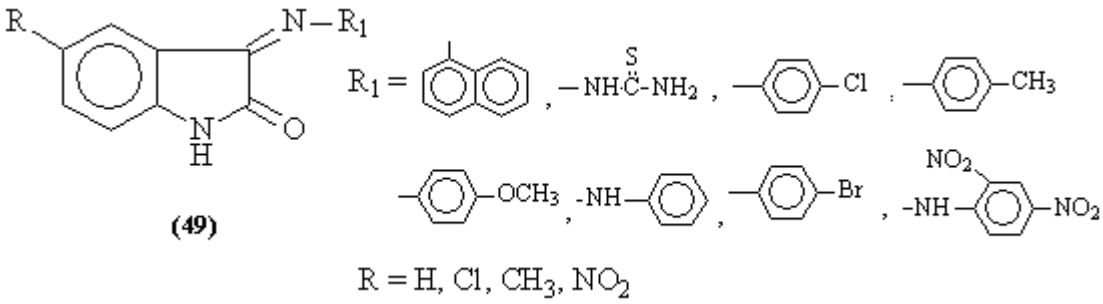
ISATINS AS ANALGESICS, ANTIPYRETICS AND ANTI-INFLAMMATORY AGENTS

Patricia Dias et *al.,* **64** designed to investigate the inhibitory effect of isatin on lipopolysaccharide/interferon-γ induced expression of inducible nitric oxide synthase (iNOS) and COX-2 **(48)**.



(48)

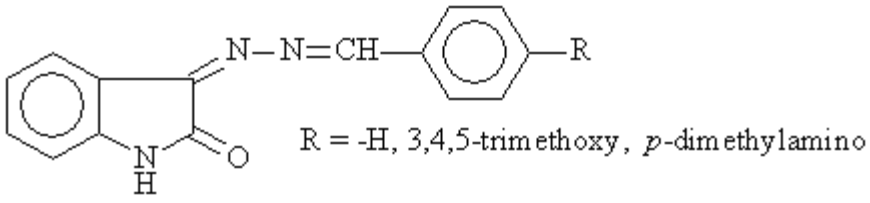
Sridhar and Ramesh [65] reported the synthesis of Schiff bases and hydrazones of isatins **(49)**. They were evaluated for analgesic, anti-inflammatory and antipyretic activities.



(49)

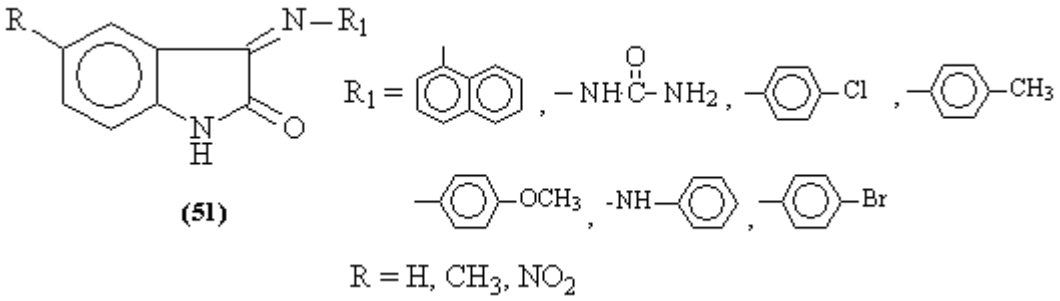
The compounds with 5-chloro group exhibited significant antipyretic activity whereas 5-methyl group exhibited moderate anti-inflammatory activity respectively.

Suroor A. Khan *et al.,* [66] reported the synthesis of Schiff bases of isatin **(50)** and they were evaluated for analgesic activity. These compounds were found to be less activity than Aspirin.



(50)

S.N. Pandeya *et al.,* [67] reported the synthesis and antimicrobial activity of some Schiff and mannich bases of isatin and its derivatives with pyrimidine. All the tested compounds were showed mild to moderate activity against bacteria.

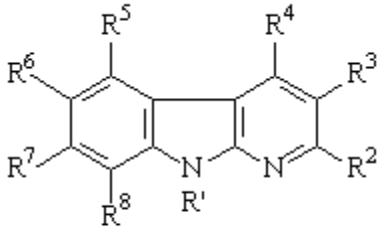


(51)

ISATIN AS CNS AGENT

Anticonvulsant activity

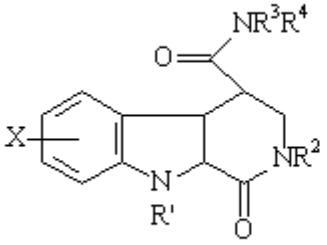
Olesen and Kanstrup[68] prepared pyrido [2, 3-b] indoles to treat a disease in the CNS *via* the metabotrophic glutamate receptor system. The title compounds are useful for treating diseases in the CNS such as epilepsy, senile dementia and Parkinsonism **(52).**



(52)

R^1 = H, C_{1-6} alkyl (CH_3, C_2H_5, C_3H_7-C_6H_{13}); C_{2-6} alkenyl (C_2H_4, C_3H_6, -C_6H_{12}), R^2 = piperidino, morpholino; R^3 = H, COOH, CN; R^4 = H, C_{1-6} alkyl (CH_3, C_2H_5, C_3H_7); R^5 = R^8 = H, NO_2, NH_2

Evanno et al., [69] synthesized 1*H*-pyrido [3, 4-b] indole-4-carboxamide derivatives **(53)**.



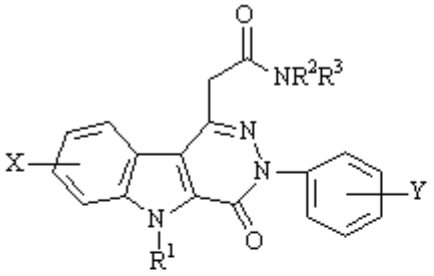
(53)

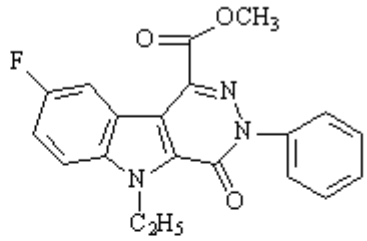
X = H, holo, axlkyl, alkoxy, CF_3, OCH_3; R^1 = H, alkyl, cyclopropyl, CH_3; R^2 = alkyl, phenyl alkyl, cyclo-hexylmethyl, thienylmethy; R^3 = R^4 = H, alkyl, 2-methoxy ethyl, OC_2H_5, carboxy alkyl, alkoxycarbonylakyl, phenyl alkyl, pyrrolidinyl, piperidinyl, morpholinyl, 4-methyl piperazinyl azetidinyl, thiadiazolinyl

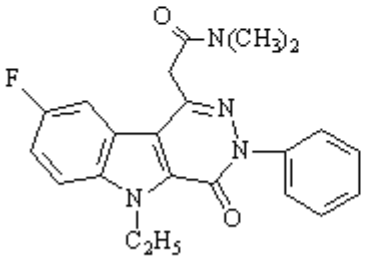
The different substituted compounds (53) were tested for their anxiolytic, hypnotic and anticonvulsant activities.

Evanno *et al.,* [70] synthesized 4-oxo-3,5–dihydro-4-*H*-pyridazano-4,5-b-indole-1-acetamide derivatives that can be used for treating diseases related to GABA aminergic transmission disorders. The compounds also shows hypnotic and

anticonvulsant activities in rats and mice. The structures of the compounds are given in **(54)**.



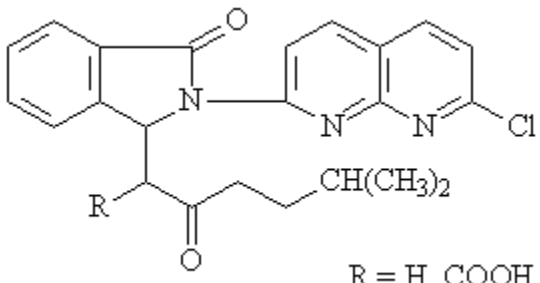




X = H, halo, CH_3, OCH_3, $OCH_2C_6H_5$; Y = H, 1 halo atom, CH_3, OH, OCH_3, NO_2; R^1 = H, C_{1-4} alkyl (CH_3, C_2H_5, C_3H_7, *etc.);* R^2R^3 = H, C_{1-4} alkyl, $CH_2C_6H_5$ or R^2R^3 = azetidine, pyrrolidinyl, 3-ethoxy pyrrolidinyl, piperidinyl, morpholinyl, 4-methyl piperazinyl, 1,3-thiadiazolinyl

(54)

David *et al.,* [71] have shown that 2-aminonapthyridine is prepared by ring cleavage of 2-isoindolinyl napthyridine **(55)**.

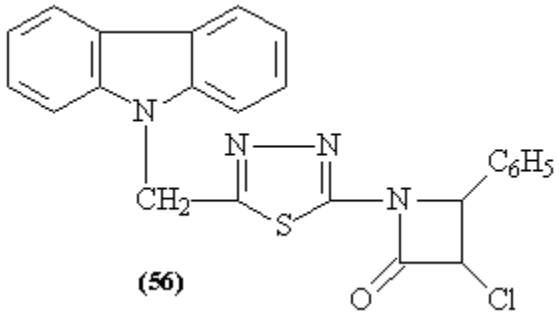


R = H, COOH

(55)

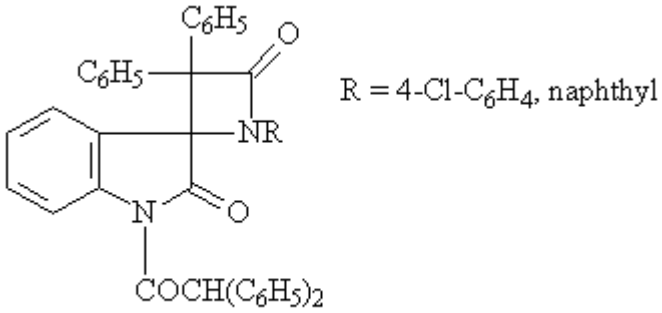
These compounds have exhibited remarkable anxiolytic, hypnotic, and anticonvulsant and muscle relaxant properties.

Srivastava *et al.,* [72] synthesized a series of compounds from carbazole, which on condensation with chloroacetyl chloride in the presence of triethylamine afforded azetidinones. Some of the compounds exhibited promising antibacterial, antifungal, anti-inflammatory and anticonvulsant activities **(56)**.



(56)

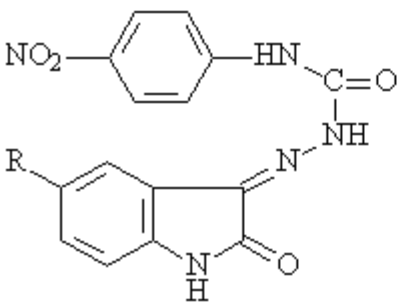
Singh *et al.,* [73] synthesized a series of isatin-based spiroazetidinones and screened them for their anticonvulsant activity **(57).**



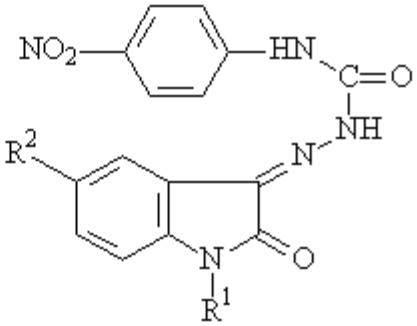
(57)

Pandeya *et al.,*[74] synthesized a series of *p*-nitro phenyl substituted semicarbazones and their anticonvulsant activity was screened against maximal electroshock (MES), subcutaneous pentylenetetrazole (ScPTZ) and subcutaneous strychnine (ScSTY) tests **(58)**. All the compounds were active in subcutaneous pentylenetetrazole and MES tests. Two compounds were active in the MES test at 100 mg kg–1.

Pandeya *et al.,* [75] synthesized a series of *N*-methyl/acetyl-5-unsubstituted-isatin-3-semicarbazones **(58)**.



R = H, Cl, NO_2

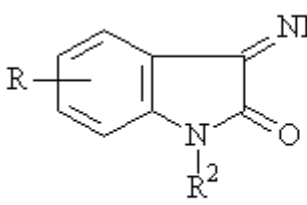


R = 2-Cl, 3-Cl, 4-Cl, 4-Br, 4-NO_2, 4-SO_2NH_2
R^1 = CH_3, $COCH_3$, R^2 = H, NO_2

(58)

In this series, compounds with 4-bromo and 2-chloro substitution (R = 4-Br and 2-Cl) showed promising activity and were also active in MES, subcutaneous pentylenetetrazole and subcutaneous strychnine induced tests.

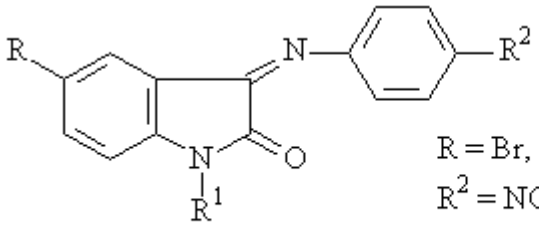
Further, Pandeya *et al.,* [76] synthesized halo substituted isatin semicarbazones to study the role of hydrogen bonding for anticonvulsant activity **(59)**.



R = H, 4-Cl, 5-Cl, 6-Cl
R^1 = -CH_2O-C_6H_5, 1-CH_2O-(4-Br)-C_6H_4
R^2 = $COCH_3$, $CH_2C_6H_5$

(59)

Pandeya *et al.,*[77] had synthesized Schiff bases of *N*-methyl and *N*-acetyl isatin derivatives with different aryl amines and screened them for anticonvulsant activity against MES and scMet. *N*-methyl-5-bromo-3-(*p*-chlorophenylimino) isatin exhibited anticonvulsant activity in MES and scMet with *LD*50 > 600 mg kg–1, showing better activity than the standard drugs such as phenytoin, carbamazepine and valproic acid **(60).**

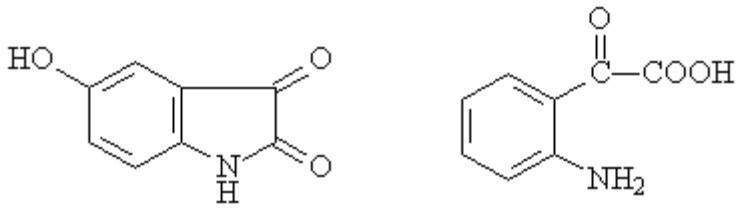


$R = Br, NO_2$; $R^1 = CH_3, COCH_3$;
$R^2 = NO_2, COOH, OCH_3, Cl, F$

(60)

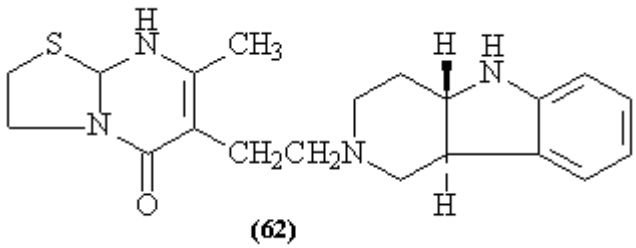
Anxiogenic and other CNS activities

Palit *et al.,* [78] studied the behavioral effects of isatin, a putative biological factor in rhesus monkeys. Isatin, one of the constituents of tribulin, a postulated endocoid marker of stress and anxiety, has been shown to induce anxiety in rodents. Medvedev *et al.,* [79] studied a range of isatin analogues for their *in vitro* inhibition of human MAO A and B. Most analogues were less potent than isatin. Hydroxylation of the aromatic ring in isatin changed the inhibitory potency in favour of MAO A, with 5-hydroxy isatin being a potent and selective MAO A inhibitor (IC_{50} 8 Δg mL^{-1}). Isatinic acid, which is formed reversibly from isatin in alkaline medium, showed no inhibition **(61).**



(61)

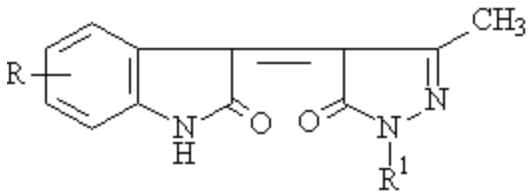
Kennis *et al.,* [80] synthesized hexahydropyrido (4, 3-b) indole derivatives.



(62)

The compound displayed in **(62)** was found to have central dopamine and serotonin antagonistic activity in the combined apomorphine, tryptamine and nor-epinephrine test in rats.

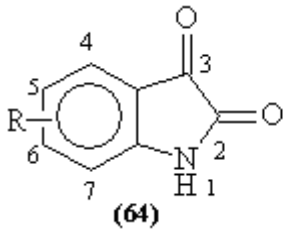
Sarangapani *et al*., [81] reported the synthesis of 3-methyl-4-(oxindol-3-ylidenyl)-5-pyrazolones **(63)**. All compounds screened for gross behavior studies they exhibited CNS depression, reduced loco motor activity. Compounds with 5-CH_3 group on indolinone and 3-methyl group on pyrazole showed antibacterial activity.



R = H, 5-CH_3, 5-Cl.
R^1 = H, C_6H_5.

(63)

Maria Eline Mathew[82] has designed to investigate the inhibitory effect of isatin derivatives on lipo polysaccharide / interferon-μ-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2- (Cox-2) proteins, production of prostaglandin E_2 (PGE_2), nitric oxide (NO), tumor necrosis factor (TNF-γ) and their capacity to scavenge NO. Isatins **(64)** inhibit TNF-α production and iNOS and COX-2 protein expression resulting on reduced levels of NO & PGE_2.

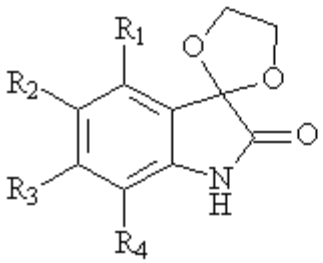


R = H, 5-F, 5-Cl, 6-Cl,
7-Cl, 4-Br, 5-I, 5-CH_3.

(64)

Giscle Zapata *et al*., [83] reported the synthesis of novel isatin ketals (nine dioxolane ketals and nine dioxane ketals) **(65)** and studied for their sedative, hypnotic and anesthetic effects using pentobarbital induced sleeping time, loco motor activity

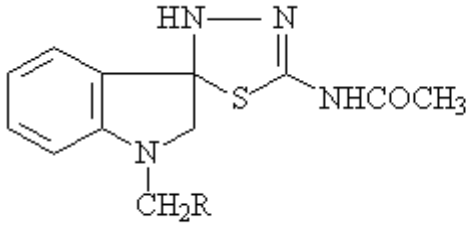
They observed dioxolane ketals were more potent than dioxane ketals for inducing sedative-hypnotic states, causing up to a three-fold increase in pentobarbital hyperosis.



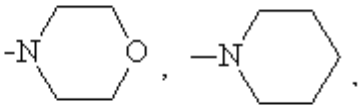
$R_1 = H, Cl, Br$
$R_2 = H, Cl, Br, F$
$R_3 = H, Br$
$R_4 = H, Cl, CF_3, OCH_3, Br$

(65)

Sasmal *et al.*, [84] reported synthesis and evaluation of CNS activity of some Spiro isationoid compounds **(66)**. All compounds exhibited CNS depression in mice.



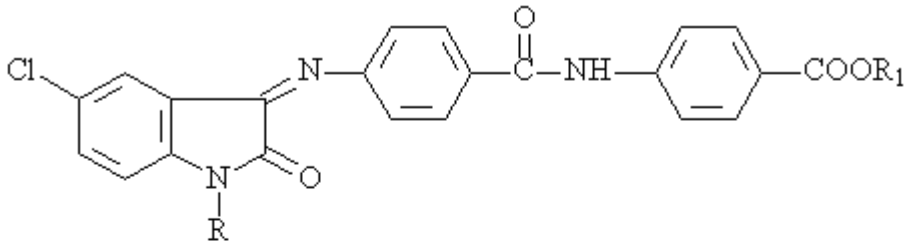
$R = -N(CH_3)_2, -N(C_2H_5)_2, -N(C_6H_5)_2,$



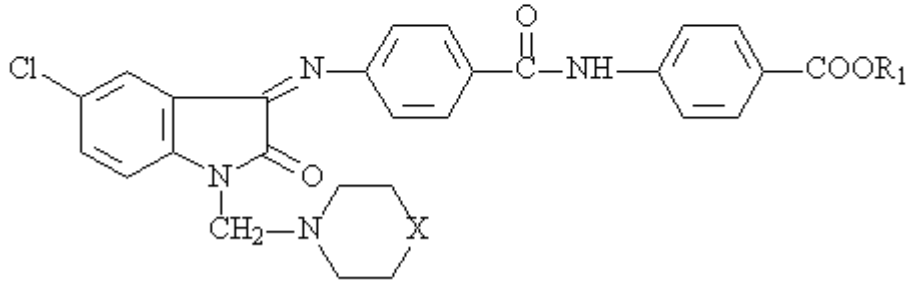
(66)

Isatins as psychotropic agents

Varma *et al.,* [85] reported the synthesis of alkyl-4-[4'-(1,2-dihydro-5-chloro-2-oxo-3H-indol-3-ylidene amino)benzoyl]aminobenzoate **(67)** and the Mannich bases of these compounds **(68)** with morpholine were found to be non-toxic and psychotropic. Some of these compounds were also active against mycobacterium tuberculosis.



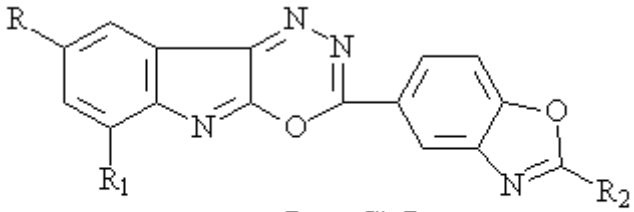
(67)



(68)

Effect of indoles on pentobarbitone induced sleeping time:

Sarangapani *et al.,* [86] reported eight new 2-substituted-[1, 3, 4] oxadiazino-[6, 5-b] indoles **(69)**. All the compounds exhibited reduction in loco motor activity and potentiating of pentobarbitone sodium induced sleeping time in experimental animals.



(69)

R = Cl, Br
R_1 = CH_3
R_2 = H, CH_3

3. AIM AND PLAN OF WORK

It is evident from literature that the presence of the indole nucleus found to have various pharmacological activities like antimicrobial, anti-convulsant, MAO inhibitory, anticancer and psychotropic activities. The very fact that one of indole derivatives (Isatins) is potential synthons, for building synthetically a variety of chemical systems known for their broader biological and pharmacological applications.

Field of chemistry of isatin remains always a fresh and even challenging for their further explorations and exploitation. It is interesting to note from the literature that the important isatin moiety is yet to be explored both synthetically and biologically in spite of the extensive work reported so far on isatins.

Therefore, keeping this in view as the main objective, the present project has been aimed at achieving the following:

- To synthesize the Isatin derivatives as depicted in Scheme-17.
- To purify the new isatin derivatives by crystallization and chromatographic techniques.
- To characterize the new compounds by physical and spectral data (IR, ^{1}H NMR and Mass)
- To screen the compounds for their biological activity by standard protocols available in literature.

Table 1: Structures of aromatic aldehydes used

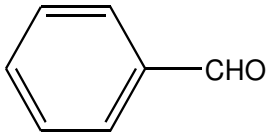
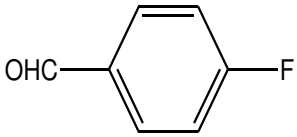
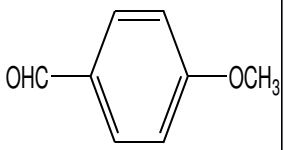
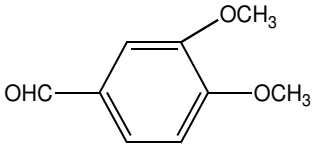
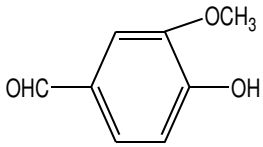
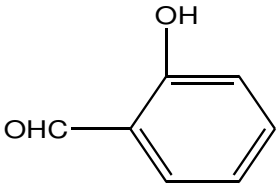
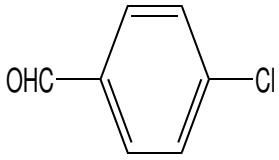
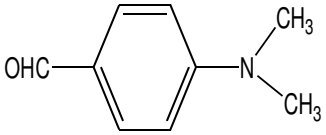
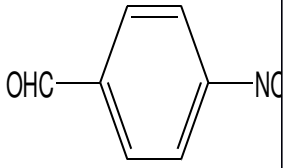
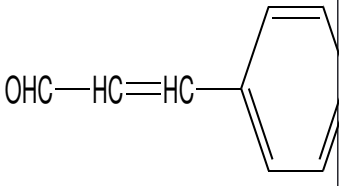
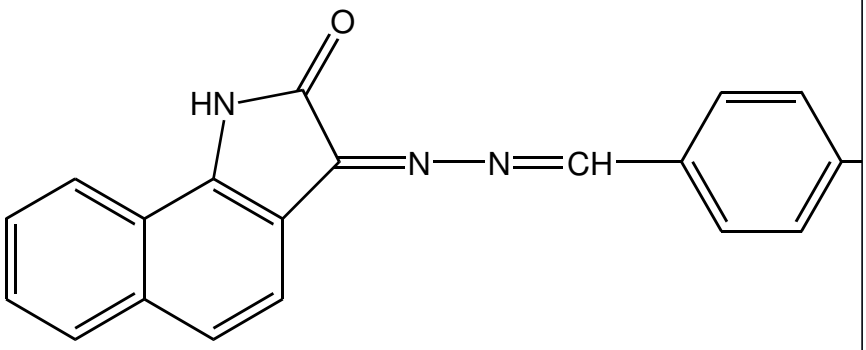
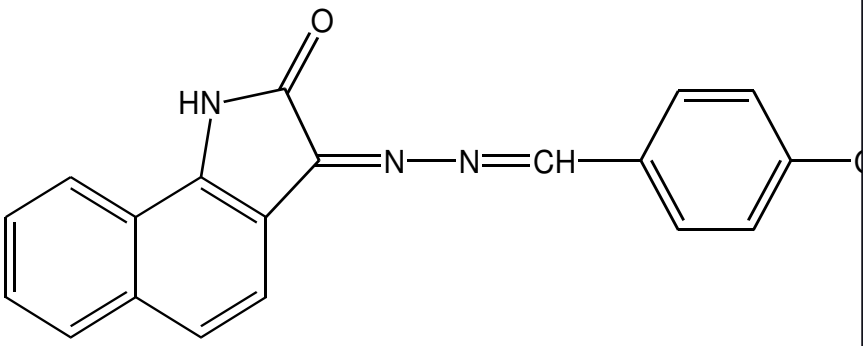
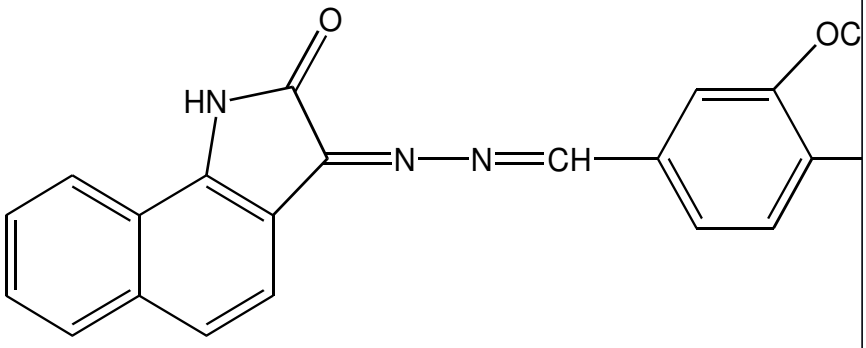
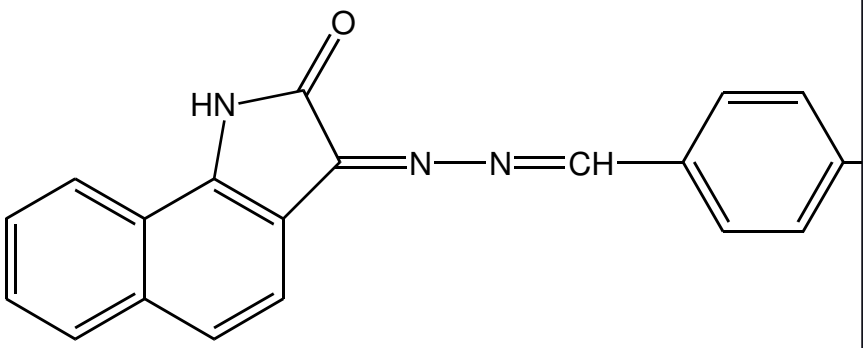
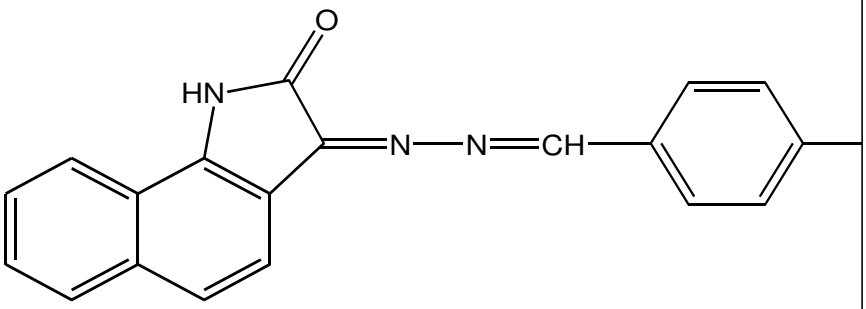
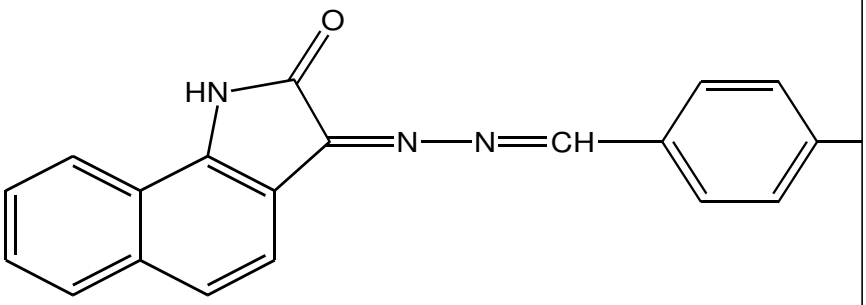
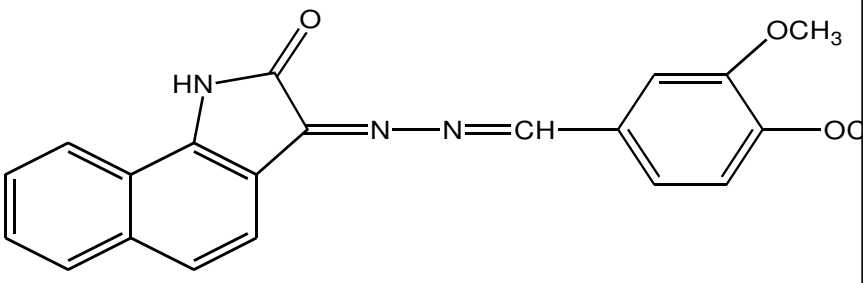
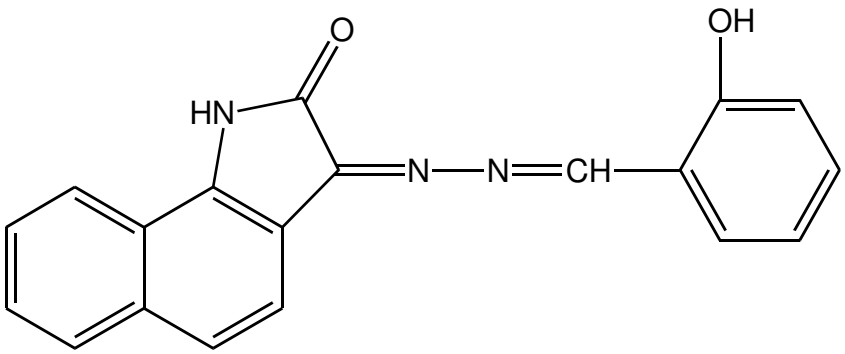
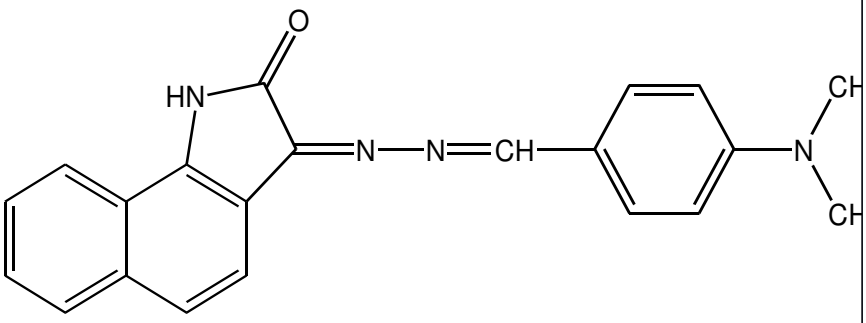
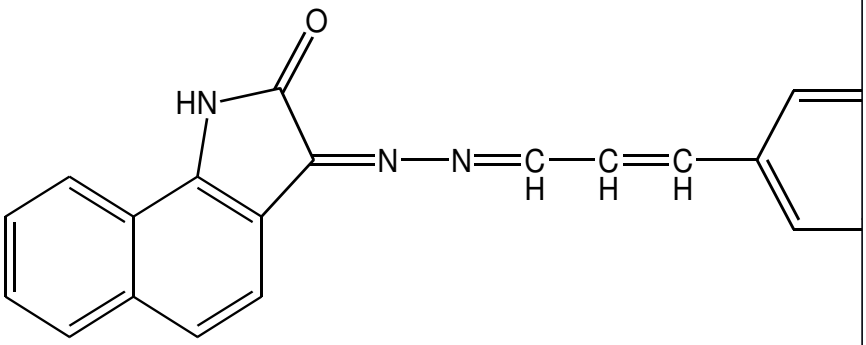
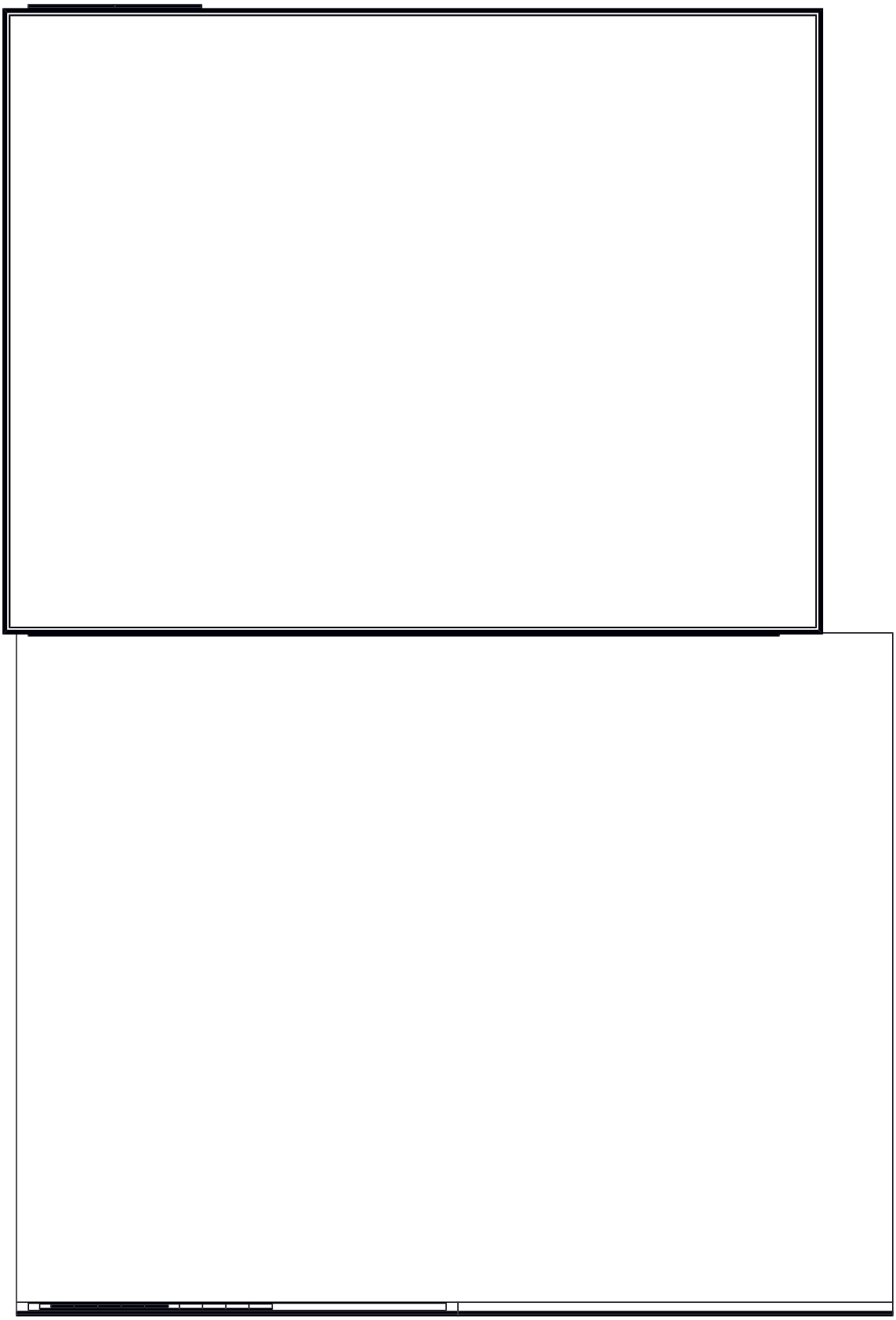
Compounds	Structures	Compounds	Structures
Va	 benzaldehyde	**Vf**	 4-fluorobenzaldehyde
Vb	 4-methoxybenzaldehyde	**Vg**	 3,4-dimethoxybenzaldehyde
Vc	 4-hydroxy-3-methoxybenzaldehyd	**Vh**	 2-hydroxybenzaldehyde
Vd	 4-chlorobenzaldehyde	**Vi**	 4-(dimethylamino)benzaldehyde
Ve	 4-nitrobenzaldehyde	**Vj**	 cinnamaldehyde

Table 2: List of compounds to be synthesized

Compounds	Structure & IUPAC names
Va	 **3-[(2-phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo [*g*]indol-2-one.**
Vb	 **3-[(2)-(4-methoxy phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one.**
Vc	 **3-[(2)-(4-hydroxy-3-methoxy phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one.**

Compounds	Structure & IUPAC names
Vd	 **3-[(2)-(4-chloro phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one**
Ve	 **3-[(2)-(4-nitro phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one.**
Vf	 **3-[(2)-(4-floro phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one.**
Vg	 **3-[(2)-(3, 4-dimethoxy phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one.**

Compounds	Structure & IUPAC names
Vh	 **3-[(2)-(2-hydroxy phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one.**
Vi	 **3-[(2)-(4-(N, N dimethyl) amino phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-one.**
Vj	 **3-[(2)-(3-phenyl allylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*] indol-2-one.**



4. EXPERIMENTAL WORK

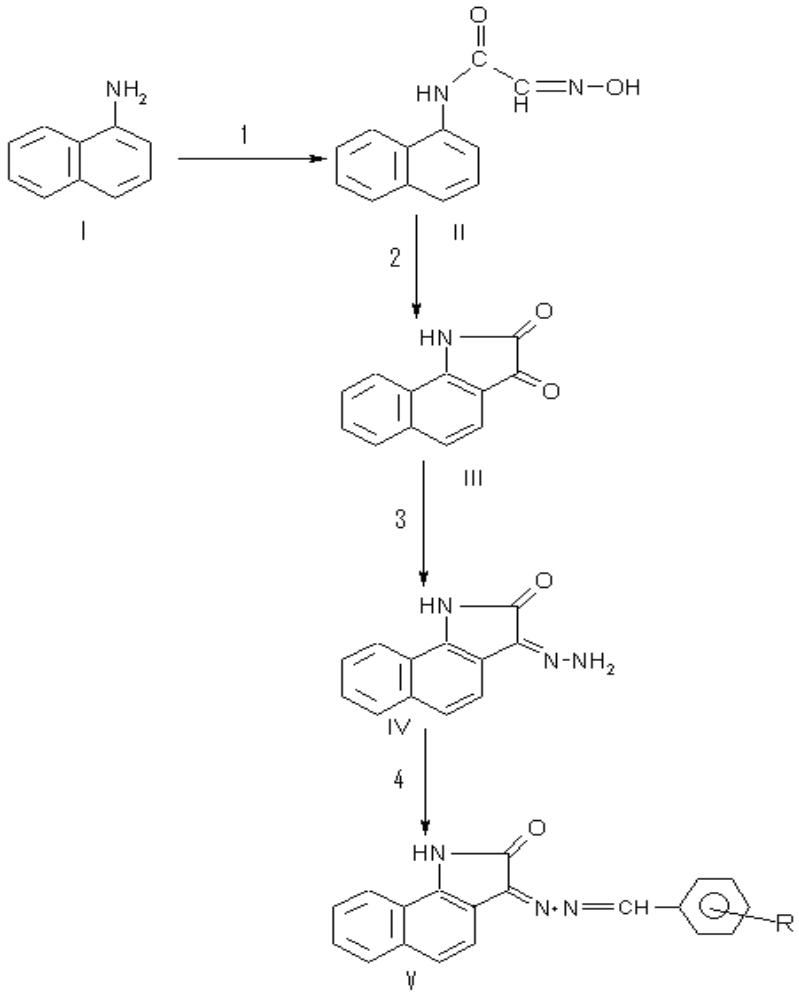
4.1 Materials and methods

The melting range of the synthesized compounds was performed by LAB INDIA visual melting point apparatus and is uncorrected. The IR spectra of the synthesized compounds were recorded on Shimdzu FT-IR spectrometer with potassium bromide pellets. Mass spectra of the synthesized compounds were performed by using the instrument Shimdzu GCMS QP 5000. The ^{1}H-NMR and spectra of the synthesized compounds were performed by using the instrument AVANCE 300 MHZ the solvent system used for the study was DMSO.

4.2 Scheme of Work

In the present investigation, involving reactions of isatin with a view to synthesize some biologically active compounds, it has been felt worth while to study the condensation reaction of 3-[(2)-(phenyl methylidene) hydrazono]-1,3-

dihydro-2*H*-benzo[*g*]indol-2-one with different aryl aldehydes, as such reactions are not reported so far, and also to evaluate the products, biologically. The synthesis of title compounds could be achieved by the **Scheme 17**.



I. α-Naphthyl amine.

II. (2)-Hydroxyimino-N-(naphthalene-1-yl) ethanamide.

III. 1H-benzo[g]indole-2, 3-dione.

IV. 3-Hydrazinylidene-1, 3-dihydro-2H-benzo[g]indole-2-one.

V. 3-[(2)-(phenyl methylidene) hydrazono]-1, 3-dihydro-2H-benzo[g]indol -2-ones.

R = H, 4-OCH_3 , 4-OH-3- OCH_3, 4-Cl , 4-NO_2, 4-F, 3,4-($OCH_3)_2$, 2-OH, 4-$N(CH_3)_2$, 1-CH=CH-

1. Chloral hydrate, hydroxyl amine hydrochloride & Sodium sulphate.
2. Conc. H_2SO_4
3. Hydrazine hydrate (99%), Methanol..
4. Various Aromatic aldehydes, methanol & few drops of glacial acetic acid

Scheme 17: Synthesis of 3-[(2)-(phenyl methylidene) hydrazono]-1, 3-dihydro-2H-benzo[g]indol-2-one derivatives.

4.3 General Procedure

STEP I - Synthesis of (2)-(hydroxyimino)-N-(naphthalene-1-yl) ethanamide (II)

In a 5 lit. R.B. flask were placed chloral hydrate 90g. (0.54 mole) and 1200 ml of water. To this solution, were then added crystallized sodiumsulphate (1300 g) followed by a solution of an alpha-Naphthyl amine (0.5 mol) (I) in 300 ml of water and concentrated hydrochloric acid (0.52 mol). Finally, a solution of 110g. Of hydroxylamine hydrochloride (1.58 mol) in 500 ml of water was added. The contents of flask were heated over a wire-gauge by a meker burner. So that vigorous boiling begins in about 45 minutes. After 1-2 minutes of vigorous boiling the reaction was complete. During the heating period itself the crystals of isonitrosoacetanilide started separating out. On cooling under the current of water the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent (s).

STEP II - Synthesis of 1H-benzo[g]indol-2, 3-dione (III)

Sulfuric acid (d. 1.84, 326 ml) was warmed to 50ºC in a one-liter R.B. flask fitted with an efficient mechanical stirrer and to this, finely powdered and 2-(hydroxyimino)-N-(naphthalen-1-yl)ethanamide (0.46 mol) (II) was added at such a

rate so as to maintain the temperature between 60 and 70º C, but not higher. External cooling was applied at this stage so that the reaction could be carried but more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80C and maintained at that temperature for 10 minutes, to complete the reaction. Then, the reaction mixture was cooled to room temperature and poured on crushed ice (2.5 kg). After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by recrystallisation from methanol.

STEP III - Synthesis of 3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one (IV)

Equimolar quantity (0.004 mol) of 1H-benzo[g]indole-2, 3-dione (III) and hydrazine were dissolved in 10 ml of warm methanol and refluxed for 30 min. After standing for approximately 24 h at room temperature, the product was separated by filtration. The compound was vaccum dried and recrystallized from warm methanol.

STEP IV - Synthesis of 3-[(2)-(phenyl methylidene) hydrazono]-1, 3-dihydro-2H-benzo[*g*]indol-2-ones (V)

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate aromatic aldehyde (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the products were separated by filtration. The products obtained were vacuum dried and recrystallized from warm methanol. The synthesized compounds were characterized by the physical and spectral data.

4.4 Chromatographic studies of synthesized compounds

Thin Layer Chromatography

Thin Layer Chromatography or TLC is a solid-liquid form of chromatography here the stationary phase is a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is inexpensive technique and quick that can be used for determine the number of components in a mixture, verify a substances identity, monitor the process of a reaction, determine appropriate condition for column chromatography, analyze the fractions obtained from column chromatography.

Materials and Methods

1. Preparation of plates

Silica gel G was mixed in a glass mortar to smooth consistency with the requisite amount of water and slurry was quickly transferred to the spreader. The mixtures have been spread over the plates in thickness of 0.2 mm and allow setting into a suitable holder and after 30 minutes, plates were dried at 120ºC, for further activation of the absorbent.

2. Sample application

About 2 mm of absorbent from the edge of plate was removed to give sharply defined edges. 2-5 µl volumes of synthesized compounds were spotted with the help of capillary tubes, just above 2 cm of the bottom of coated plates.

3. Development chamber

The chromatographic chamber was lined with filter paper dipping into mobile phase so as to maintain the atmospheric saturation with solvent vapors in the chamber. The solvent front was allowed to rise to distance of about 12 cm from the base line on the plate was removed from the tank and allowed to dry in the air.

4. Solvent system

The choice of best developing solvent is one of the most important decisions in practical TLC by review of literature survey on by knowing nature of compounds, this solvent system used is chloroform: ethyl acetate (9:1).

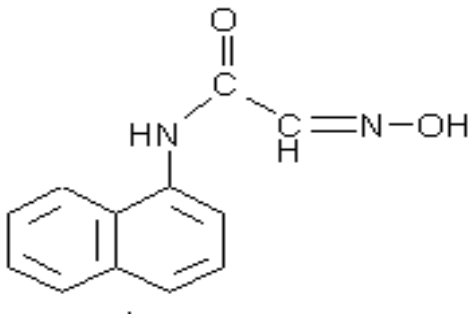
5. Detection of components

The spots were visualized under Iodine chamber.

4.5 Physico – chemical and spectral data of synthesized compounds

Using above procedure, 10 compounds (Va-Vj) were synthesized and their physic-chemical and spectral data has given below,

Spectral data of (2)-(hydroxyimino)-N-(naphthalen-1-yl)ethanamide (II).



Molecular formula : $C_{12}H_{10}N_2O_2$

Molecular weight : 214

Melting point : 175-176ºC

Solubility : Methanol, Ethanol.

TLC R_f (solvent) : 0.4 (Chloroform: Ethyl acetate; 9:1)

IR (KBr) Cm^{-1} : Naphthalene(1571.91),

C-N(876.79),

N-H(3166.10),

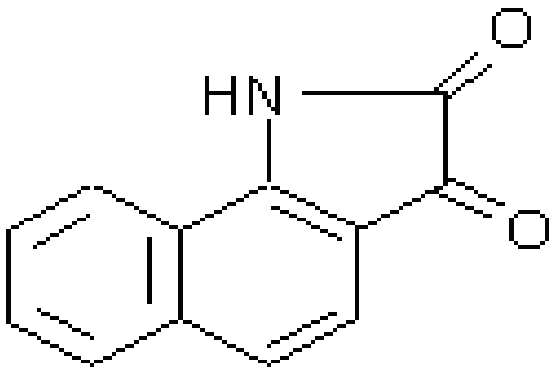
C=O(1682.58),

C=N(1571.91),

O-H(1036.86).

Mass spectra : Molecular ion ($M^{\cdot +}$) at m/z = 214

Spectral data of 1H-benzo[g]indol-2, 3-dione (III).



Molecular formula : $C_{12}H_7NO_2$

Molecular weight : 197

Melting point : 183ºC

Solubility : Methanol, Ethanol.

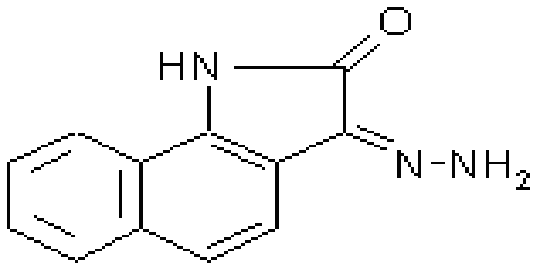
TLC R_f (solvent) : 0.5 (Chloroform: Ethyl acetate; 9:1)

IR (KBr) Cm^{-1} : Naphthalene(1587.11),

C-N(1387.48),

		N-H(3195.58),
		C=O(1717.33).
^{1}H NMR	:	6.6-6.9 (m , 6H, Ar-H)
		7.2 (s,1H, NH)
Mass spectra	:	Molecular ion ($M^{\cdot +}$) at m/z = 197

Spectral data of 3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one (IV)



Molecular formula : $C_{12}H_9N_3O$

Molecular weight : 211

Melting point : 192-194ºC

Solubility : Methanol, Ethanol.

TLC R_f (solvent) : 0.45 (Chloroform: Ethyl acetate; 9:1)

IR (KBr) Cm^{-1} : Naphthalene (1587.11),

C-N (1387.43),

N-H (3195.58),

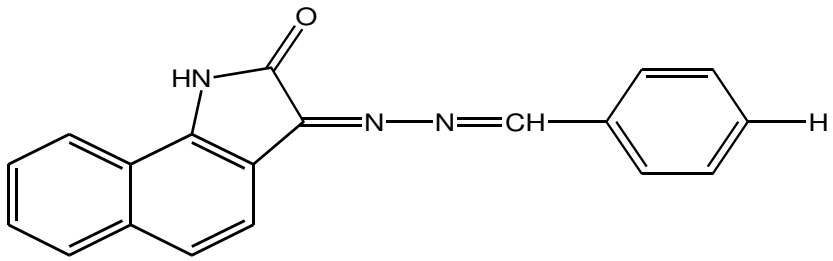
C=O (1720.08),

C=N (1623.98),

N-H (2918.07).

SAMPLE - Va

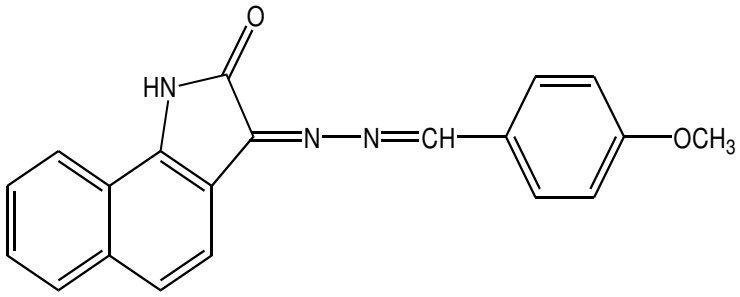
Equimolar quantity (0.01mol) of 3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of benzaldehyde (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (orange colored) is characterized by the physical and spectral data.



Molecular formula	:	$C_{19}H_{13}ON_3$
Molecular weight	:	299.32
Melting point	:	140-142C
Yield	:	67%w/w
Solubility	:	Methanol, Ethanol.
TLC R_f (solvent)	:	0.82 (Chloroform: Ethyl acetate; 9:1)
IR(KBr) cm^{-1}	:	Naphthalene (1550.03), N-H (3190.58), C=O (1748.03), C=N (1620.76), Phenyl (845.78), C-H(2924.25).
^{1}H NMR(o ppm)	:	10.420 (1H, s), 3.763 (2H, bs, $2^{\delta}NH_2$), 1.246 (4H, pyrrolic protons), 2.993 (4H, protons), 7.068 (1H, s, C- 5H), 6.004 -8.47 (10 H, aromatic protons).

SAMPLE – Vb

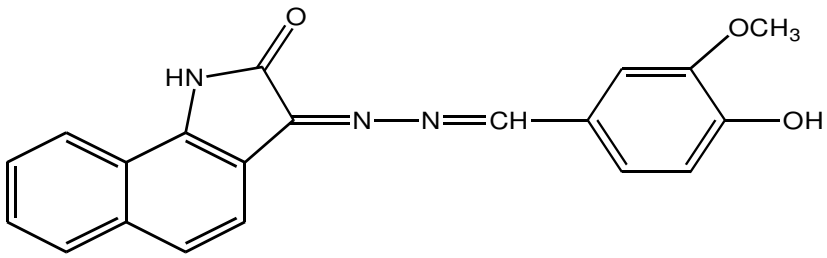
Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of 4-methoxy benzaldehyde (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (reddish brown colored) is characterized by the physical and spectral data.



Molecular formula	:	$C_{20}H_{15}O_2N_3$
Molecular weight	:	329.35
Melting point	:	146°C
Yield	:	54%w/w
Solubility	:	Methanol, Ethanol.
TLC R_f (solvent)	:	0.68 (Chloroform: Ethyl acetate; 9:1)
IR(KBr) cm^{-1}	:	Naphthalene (1565.08), N-H (3198.05), C=O (1750.09), C=N (1641.11), Phenyl (836.10), C-O-C (1260.29), Methyl C-H(2857.04).
^{1}H NMR(o ppm)	:	1.248 & 2.511 (4H pyrolic protons), 3.305 (2H, s, NH), 7.036 (1H, s, C-5H), 10.420 (methoxy).

SAMPLE – Vc

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of 4-hydroxy-3-methoxy benzaldehyde (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (pale brown colored) is characterized by the physical and spectral data.



Molecular formula : $C_{20}H_{15}O_3N_3$

Molecular weight : 345.35

Melting point : 130-131$^{\delta}$C

Yield : 91%w/w

Solubility : Methanol, Ethanol.

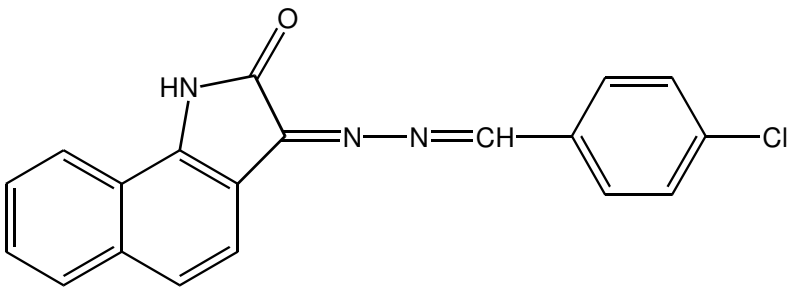
TLC R_f (solvent) : 0.57 (Chloroform: Ethyl acetate; 9:1)

IR(KBr) cm^{-1} : Naphthalene (1559.18), N-H (3039.33), C=O (1739.08), C=N (1640.24), Phenyl (810.30), C-O-C (1283.47), Methyl C-H(2923.99), O-H(3435.06).

^{1}H NMR(o ppm) : 7.114 (1H, s, C-5H), 10.420 (1H,phenolic), 1.248 (H, pyrolic protons), 3.301 (H, 2$^{\delta}$ NH).

SAMPLE – Vd

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of 4-chloro benzaldehyde(0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (bluish red colored) is characterized by the physical and spectral data.



Molecular formula : $C_{19}H_{12}OCl\,N_3$

Molecular weight : 333.77

Melting point : 132-134^{o}C

Yield : 48%w/w

Solubility : Methanol, Ethanol.

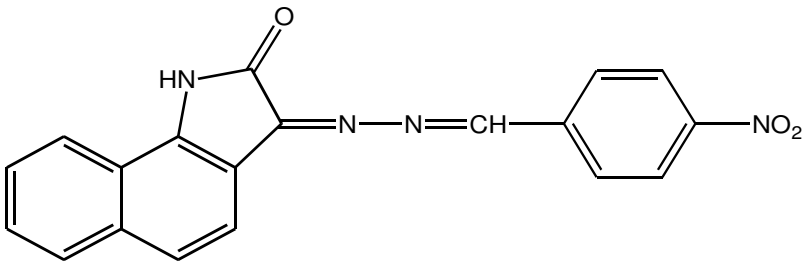
TLC R_f (solvent) : 0.64 (Chloroform: Methanol; 9:1)

IR(KBr) cm^{-1} : Naphthalene (1581.10), N-H (3096.28), C=O (1760.52), C=N (1634.34), Phenyl (880.81), C-Cl(760.26).

^{1}H NMR(o ppm) : 7.150 (1H, s, C-5H), 1.247 (H, pyrolic protons), 3.327(H, 2^{δ} NH).

SAMPLE – Ve

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2h-benzo[g]indol-2-one (iv), an appropriate quantity of 4-nitro benzaldehyde (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (yellowish red colored) is characterized by the physical and spectral data.



Molecular formula : $C_{19}H_{12}O N_4$

Molecular weight : 344.32

Melting point : 152-154°C

Yield : 76%w/w

Solubility : Methanol, Ethanol.

TLC R_f (solvent) : 0.48 (Chloroform: Ethyl acetate; 9:1)

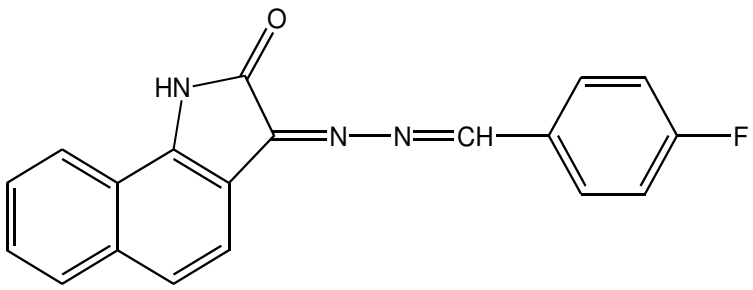
IR(KBr) cm^{-1} : Naphthalene (1599.32), N-H (3956.36), C=O (1740.27), C=N (1645.66), Phenyl (824.81), Ar-NO_2 (1384.61, 1444.16), C-N(719.98).

^{1}H NMR(o ppm) : 1.241 & 2.514 (H, pyrrolic protons), 3.315 (1H, 2^{δ} NH),

7.313 (1H, s, C-5H), 6.814-8.403 (9H, aromatic protons).

SAMPLE - Vf

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of 4-floro benzaldehyde (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (crimson red colored) is characterized by the physical and spectral data.



Molecular formula : $C_{19}H_{12}OFN_3$

Molecular weight : 317.31

Melting point : 171°C

Yield : 92%w/w

Solubility : Methanol, Ethanol.

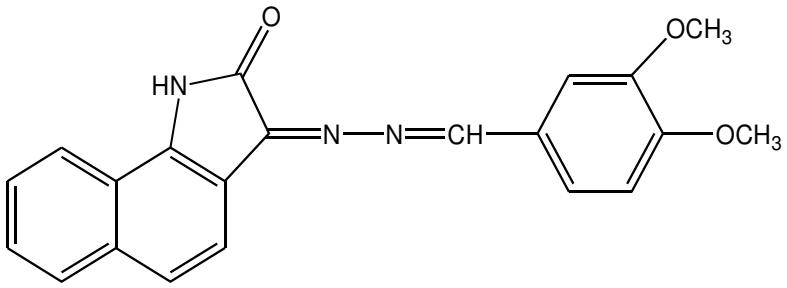
TLC R_f (solvent) : 0.73 (Chloroform: Ethyl acetate; 9:1)

IR(KBr) cm^{-1} : Naphthalene (1549.98), N-H (3192.62), C=O (1752.21), C=N (1636.63), Phenyl (840.28), C-F(1233.74).

^{1}H NMR(o ppm) : 1.189 & 2.902 (H, benzylic protons), 3.563 (1H, 2^{δ} NH), 7.088 (1H, s, C-5H).

SAMPLE – Vg

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one(IV),an appropriate quantity of 3,4-dimethoxy benzaldehyde(0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (reddish brown colored) is characterized by the physical and spectral data.



Molecular formula : $C_{21}H_{17}O_3N_3$

Molecular weight : 359.37

Melting point : 124-126°C

Yield : 46%w/w

Solubility : Methanol, Ethanol.

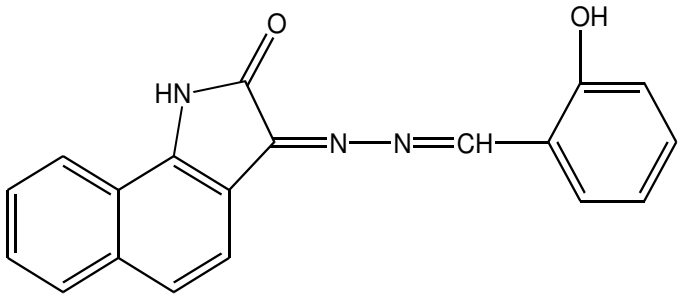
TLC R_f (solvent) : 0.76 (Chloroform: Ethyl acetate; 9:1)

IR(KBr) cm^{-1} : Naphthalene (1562.10), N-H (3184.10), C=O (1738.26), C=N (1622.10), Phenyl (815.80), C-O-C (1259.76, 1249.16), C-H(2923.38)..

^{1}H NMR(o ppm) : 8.317 (3H, s, methoxy protons), 1.244 & 2.510 (H, pyrrolic and benzylic), 3.304 (1H, 2$^{\delta}$NH), 6.648 (1H, s, C-5H), 6.648-8.317 (8H, aromatic protons).

SAMPLE – Vh

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of 2-hydroxy benzaldehyde(0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (orange colored) is characterized by the physical and spectral data.



Molecular formula : $C_{19}H_{13}O_2N_3$

Molecular weight : 315.32

Melting point : 146^{o}C

Yield : 72%w/w

Solubility : Methanol, Ethanol.

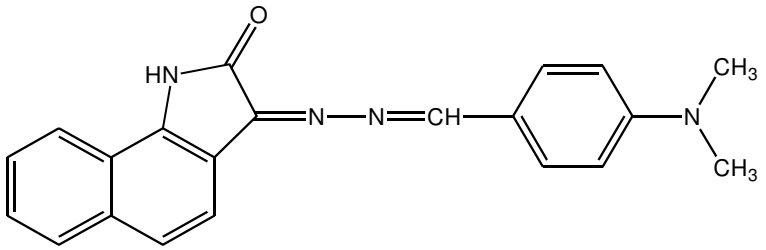
TLC R_f (solvent) : 0.84 (Chloroform: Ethyl acetate; 9:1)

IR(KBr) cm^{-1} : Naphthalene (1559.18), N-H (3039.33),

C=O (1739.08), C=N (1640.24),
Phenyl (810.30), C-OH(3435.06).

SAMPLE – Vi

Equimolar quantity (0.01 mol) of 3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of 4-(N,N-dimethyl)amino benzaldehyde(0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (dark red colored) is characterized by the physical and spectral data.



Molecular formula : $C_{21}H_{18}N_4O$

Molecular weight : 342

Melting point : 158^0C

Yield : 94%w/w

Solubility : Methanol, Ethanol.

TLC R_f (solvent) : 0.61 (Chloroform: Ethyl acetate; 9:1)

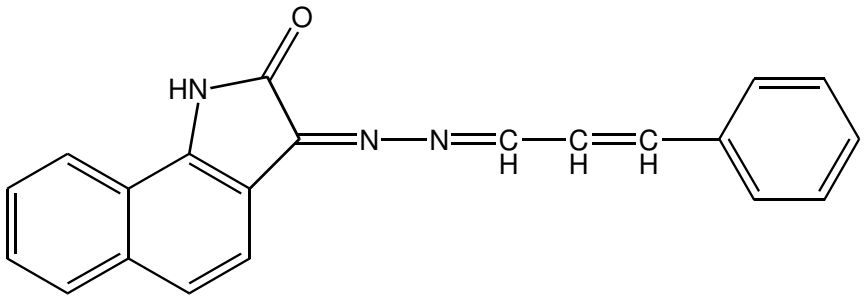
IR (KBr) Cm^{-1} : Naphthalene (1582.18), N-H (3080.63),
C=O (1726.11), C=N (1621.58), Phenyl (810.09),
t-amino (2921.85), C-N(945.57), Methyl C-H (2872.42).

^{1}H NMR(o ppm) : 2.913 & 2.513 (3H, s, methyl protons),
6.766 & 6.746 (1H, s, C-5H),
7.212-7.357 (8H, aromatic protons).

Mass spectra : Molecular ion ($M^{\cdot +}$) at m/z = 343

SAMPLE – Vj

Equimolar quantity (0.01mol) of 3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate quantity of cinnamaldehyde (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4 h. After standing for approximately 24 h at room temperature, the product is separated by filtration. The product obtained is vaccum dried and recrystallized from warm methanol. The synthesized compound (light orange colored) is characterized by the physical and spectral data.



Molecular formula : $C_{21}H_{15}ON_3$

Molecular weight : 325.13

Melting point : 148-150$^{\delta}$C

Yield : 64%w/w

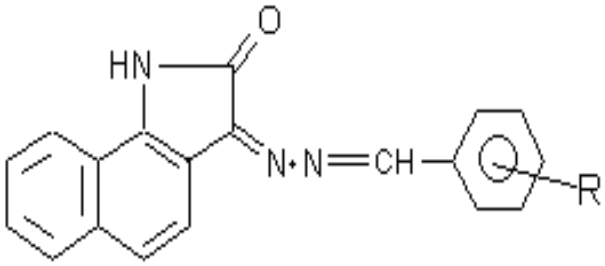
Solubility : Methanol, Ethanol.

TLC R_f(solvent) : 0.68 (Chloroform: Ethyl acetate; 9:1)

IR(KBr) cm^{-1} : Naphthalene (1557.28), N-H (3431.44), C=O (1739.46), C=N (1635.10), Phenyl (888.33), Allyl (1650.28).

^1H NMR(o ppm) : 10.431 (1H, s), 1.241 & 2.518 (2H,pyrrolic and benzylic), 3.314 (1H, 2^{δ}NH), 7.798 (1H, s, C-5H). 7.117-9.177 (9 H, aromatic protons).

Table 3: Physical data of 3-[(2-phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-ones (v)



S.No	Compds	Substituents R	Mol. Formula	Mol. Weight	M.P. (°C)	Yield (%)w/w	R_f value
1.	Va	H	$C_{19}H_{13}N_3O$	299.32	140-142	67	0.82
2.	Vb	4-OCH_3	$C_{20}H_{15}N_3O_2$	329.35	146	54	0.68
3.	Vc	4-OH, 3-OCH_3	$C_{20}H_{15}N_3O_3$	345.35	130-131	91	0.57
4.	Vd	4-Cl	$C_{19}H_{12}ClN_3O$	333.77	132-134	48	0.64
5.	Ve	4-NO_2	$C_{19}H_{12}N_4O$	344.32	152-154	76	0.48
6.	Vf	4-F	$C_{19}H_{12}FN_3O$	317.31	171	92	0.73
7.	Vg	3-OCH_3, 4-OCH_3	$C_{21}H_{17}N_3O_3$	359.37	124-126	46	0.76
8.	Vh	2-OH	$C_{19}H_{13}N_3O_2$	315.32	145-146	72	0.84
9.	Vi	4-$N(CH_3)_2$	$C_{21}H_{18}N_4O$	342.19	158	94	0.61
10.	Vj	1-CH=CH-	$C_{21}H_{15}N_3O$	325.13	148-150	64	0.68

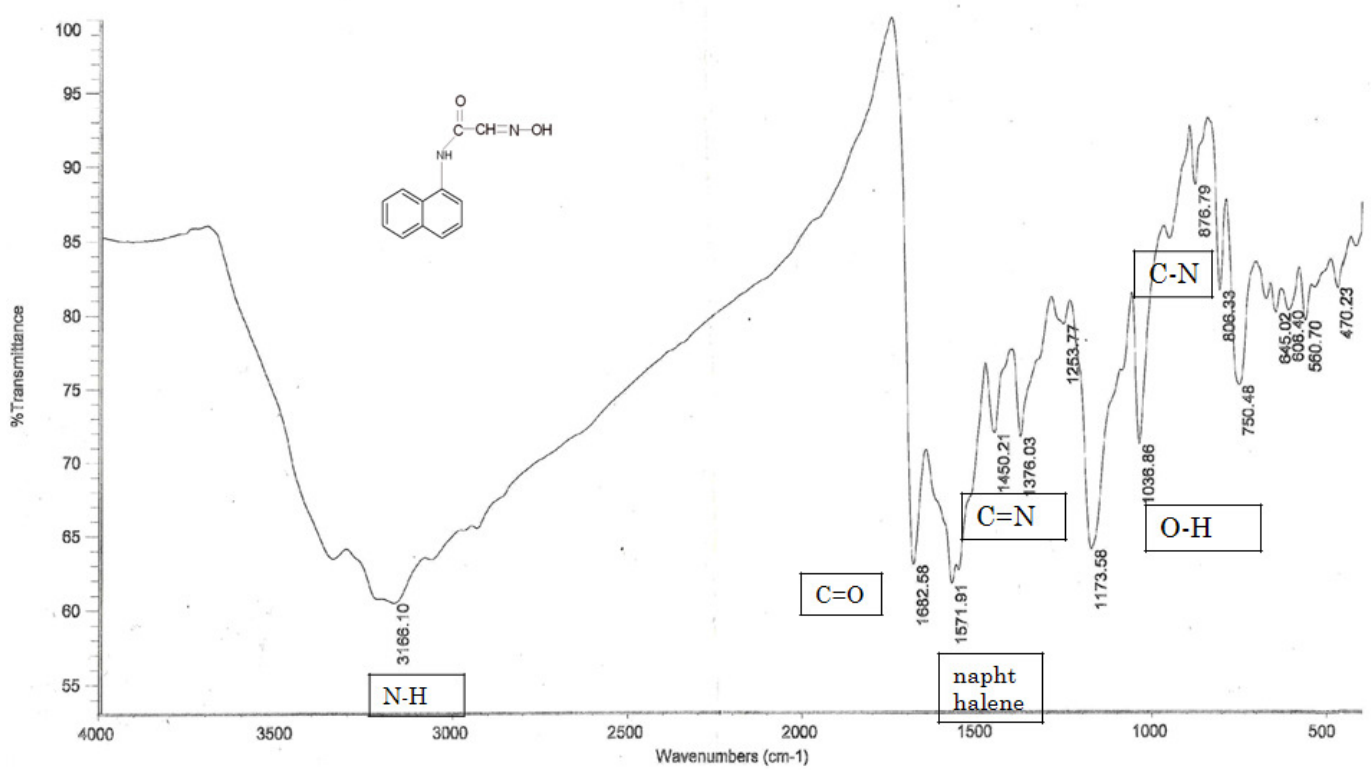


Fig. No. 2. FT-IR Spectrum of 2-(hydroxy imino)-N-(naphthalen-1-yl) ethanamide(II)

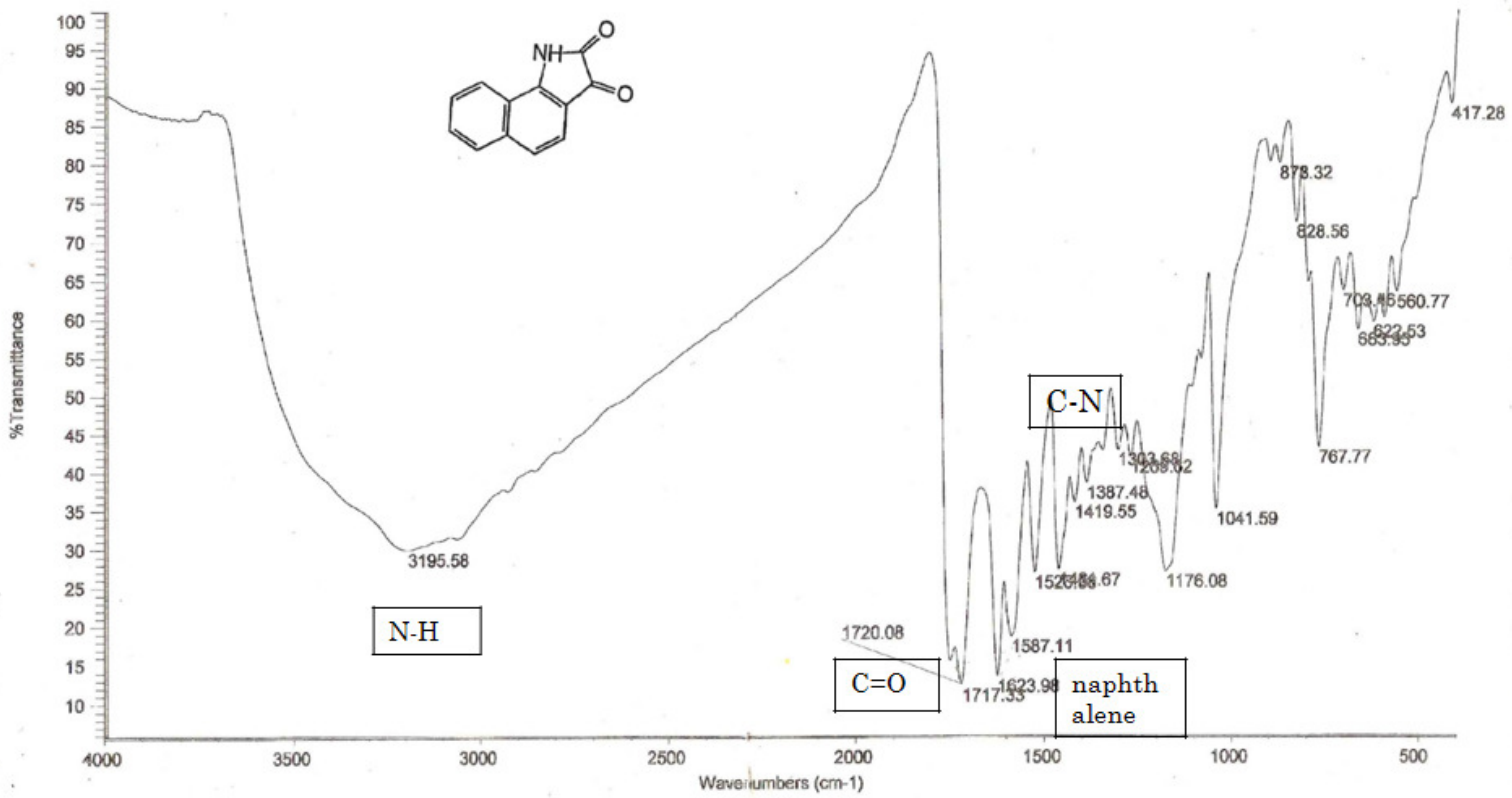


Fig. No. 3. FT-IR Spectrum of 1H-benzo[g]indole-2, 3-dione (III)

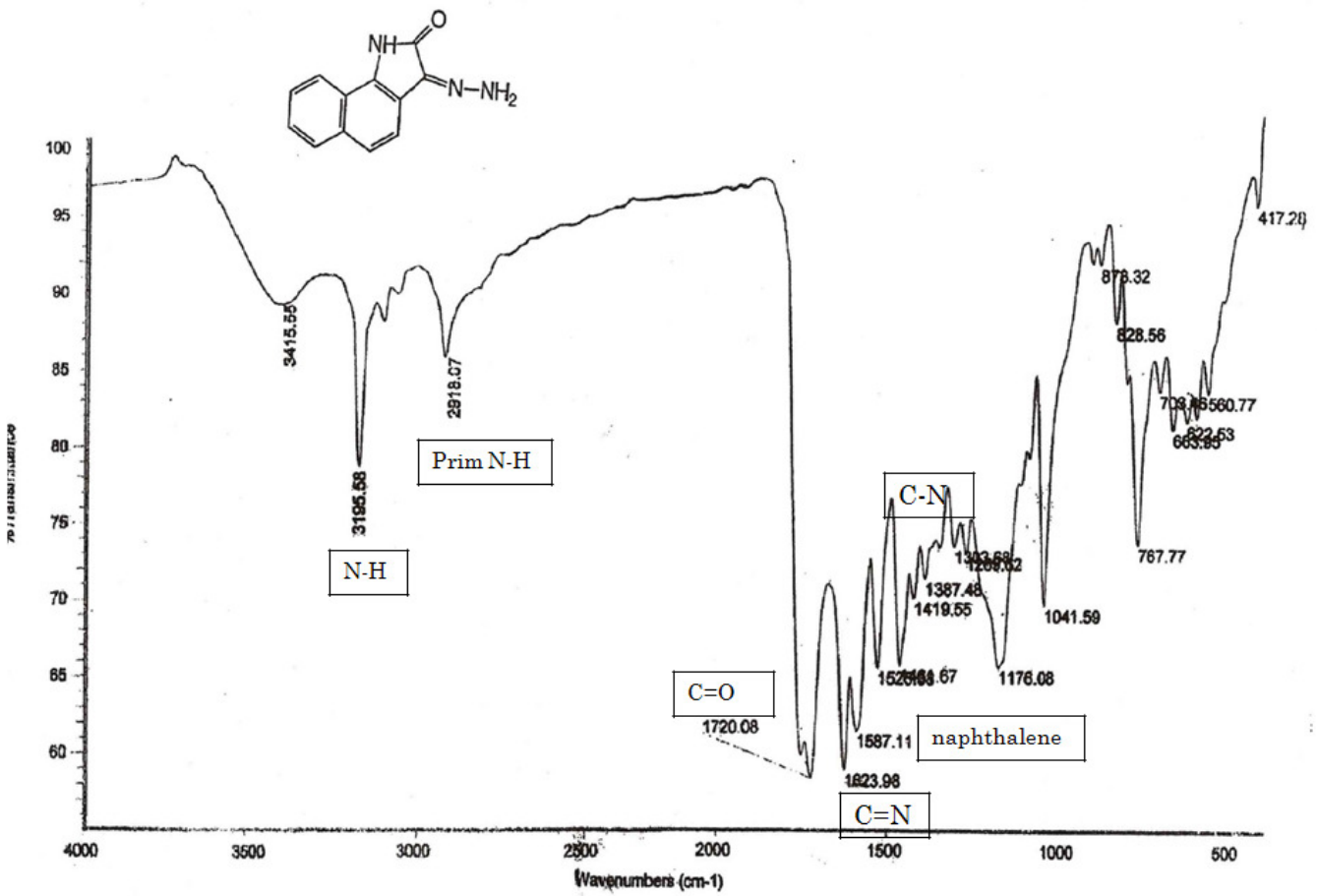


Fig. No. 4. FT-IR Spectrum of (3)-hydrazinylidene-1, 3-dihydro-2H-benzo [g] indol-2-one (IV)

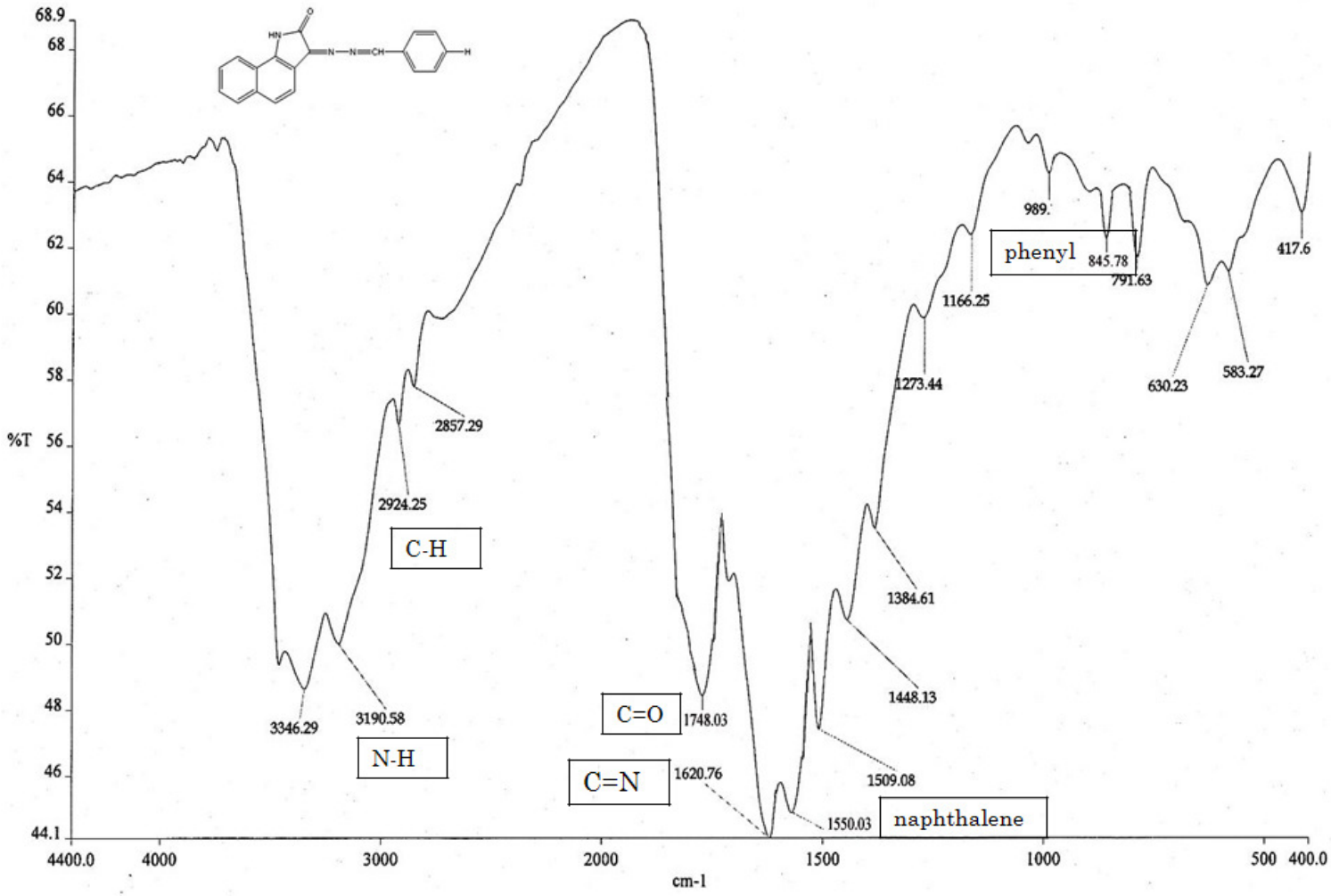


Fig. No. 5. FT-IR Spectrum of Sample Va

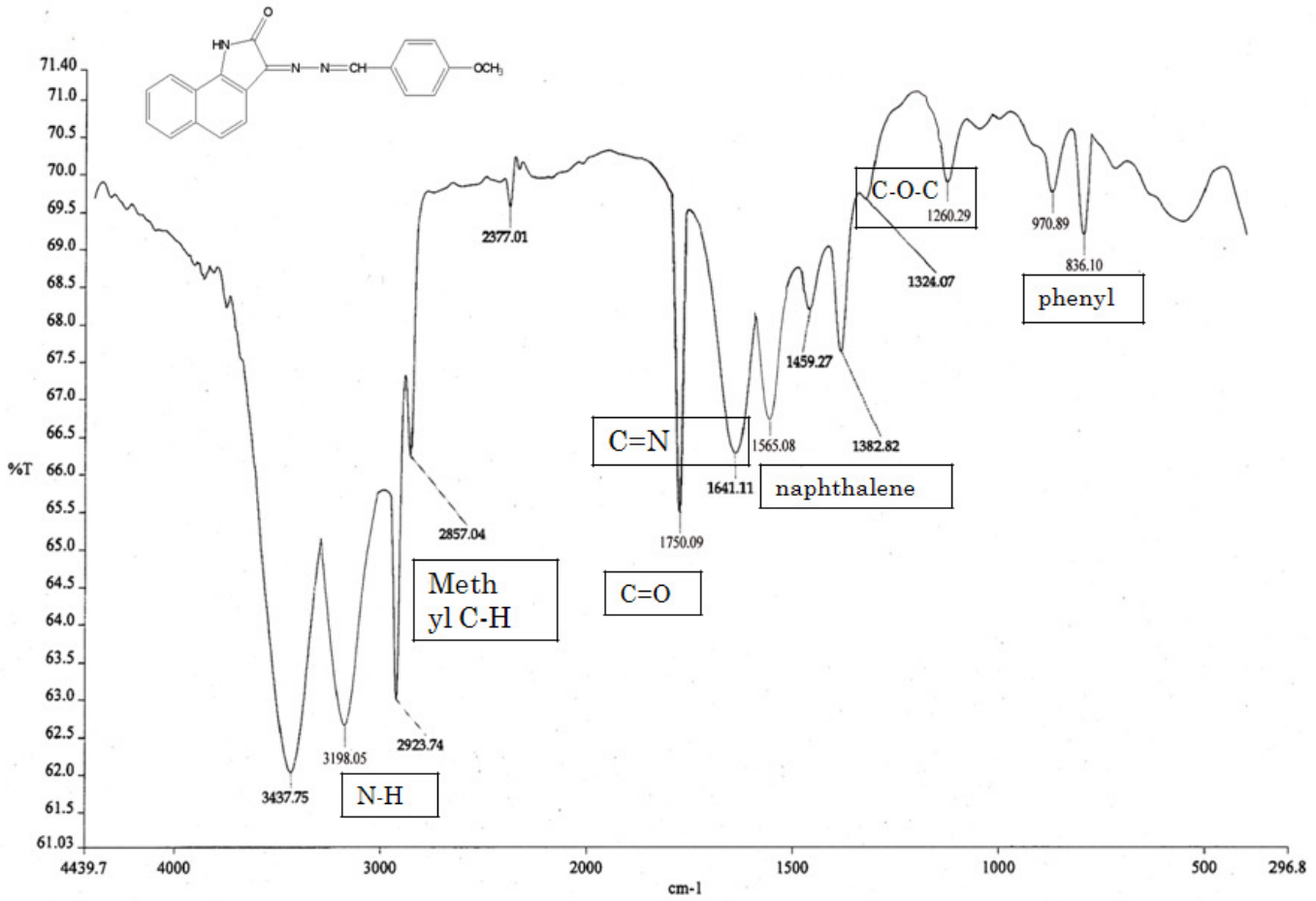


Fig. No. 6. FT-IR Spectrum of Sample Vb

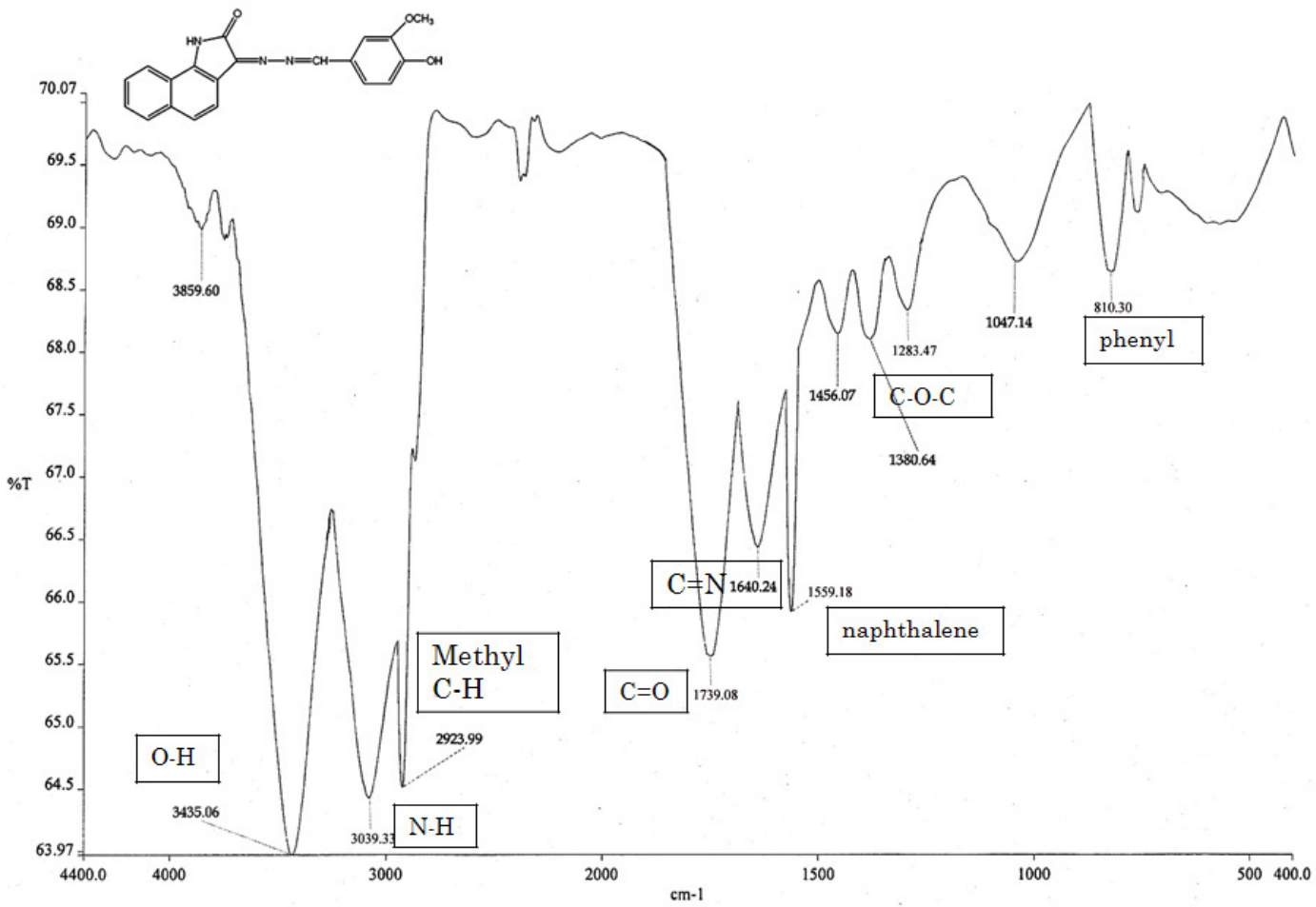


Fig. No. 7. FT-IR Spectrum of Sample Vc

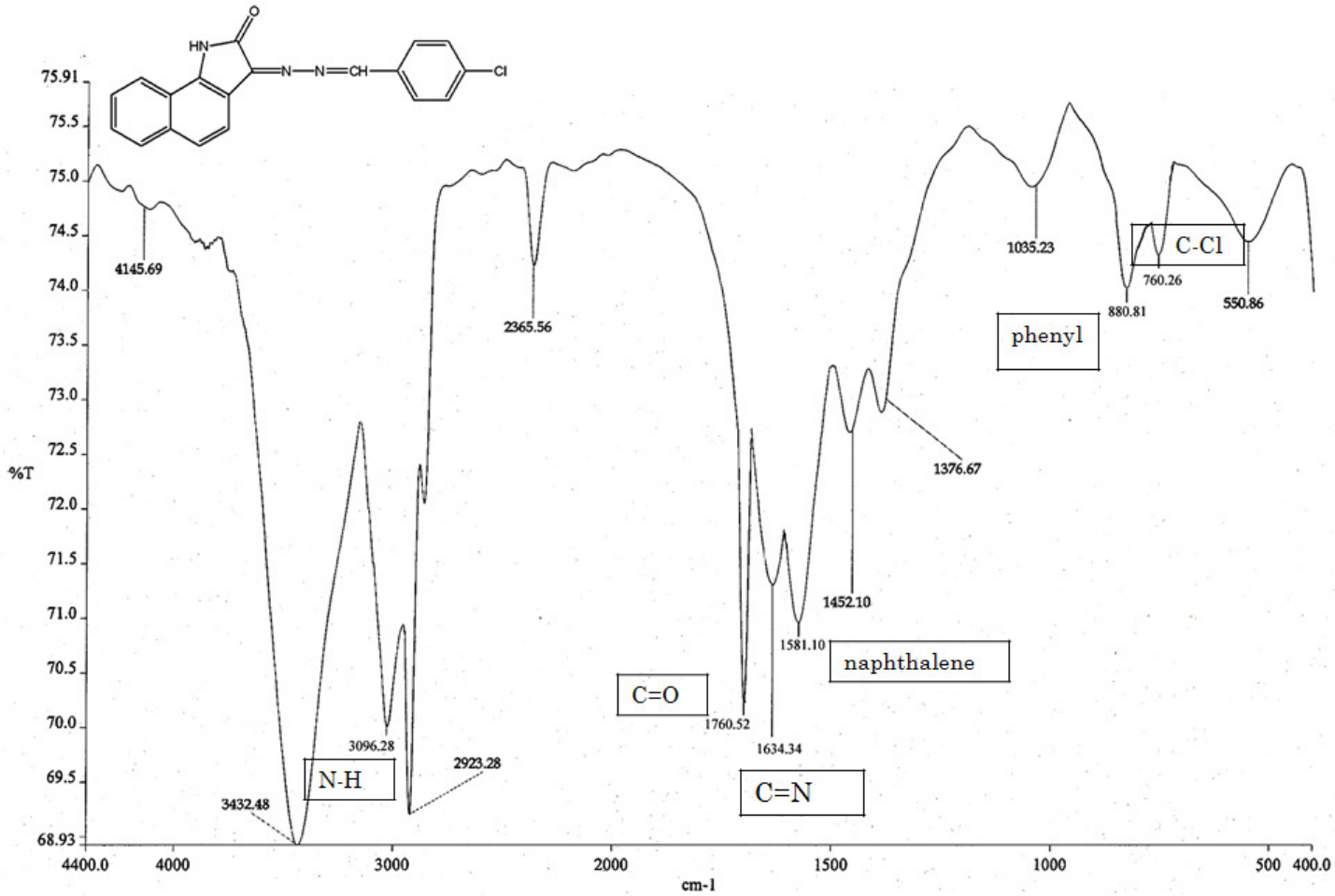


Fig. No. 8. FT-IR Spectrum of Sample Vd

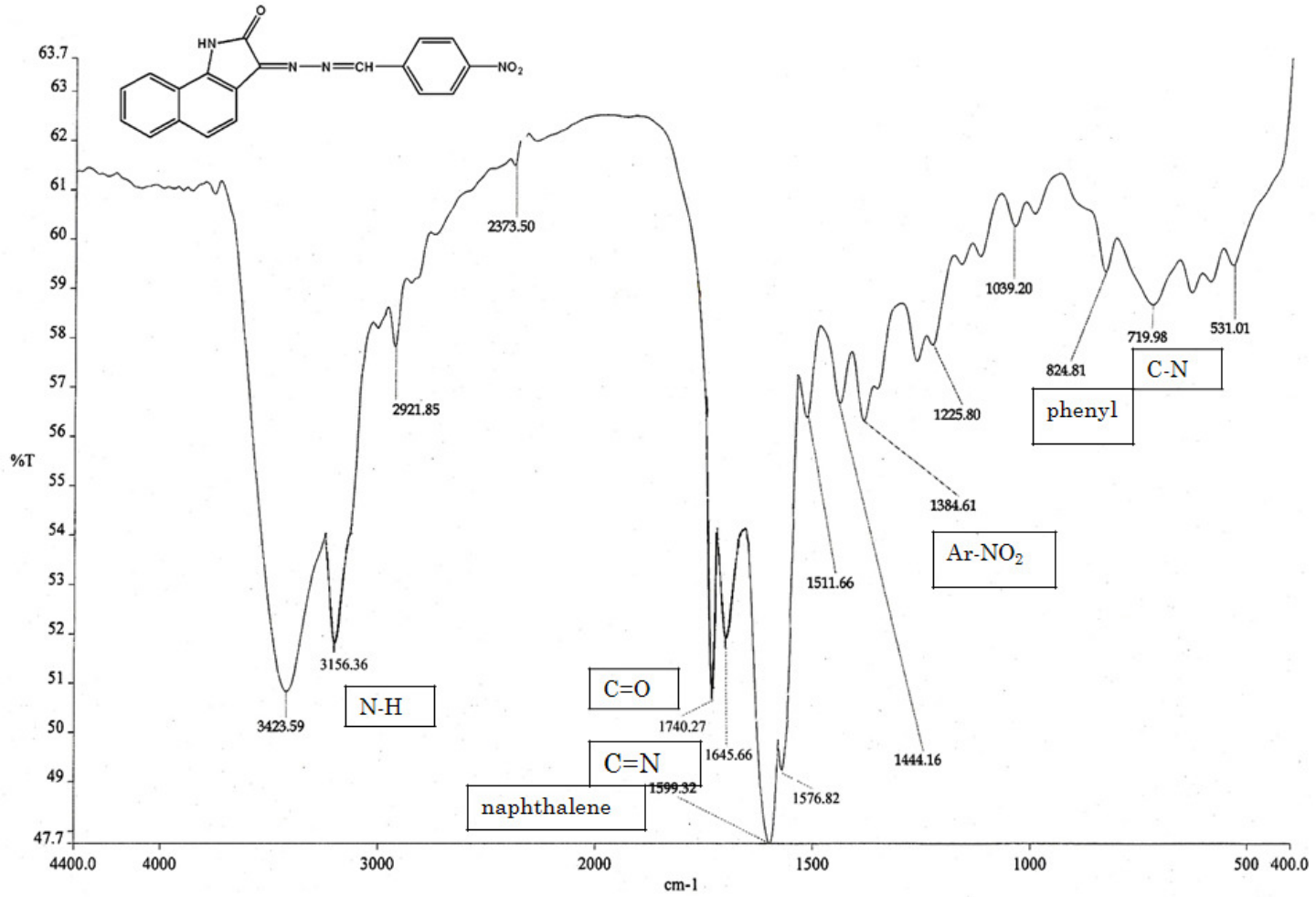


Fig. No. 9. FT-IR Spectrum of Sample Ve

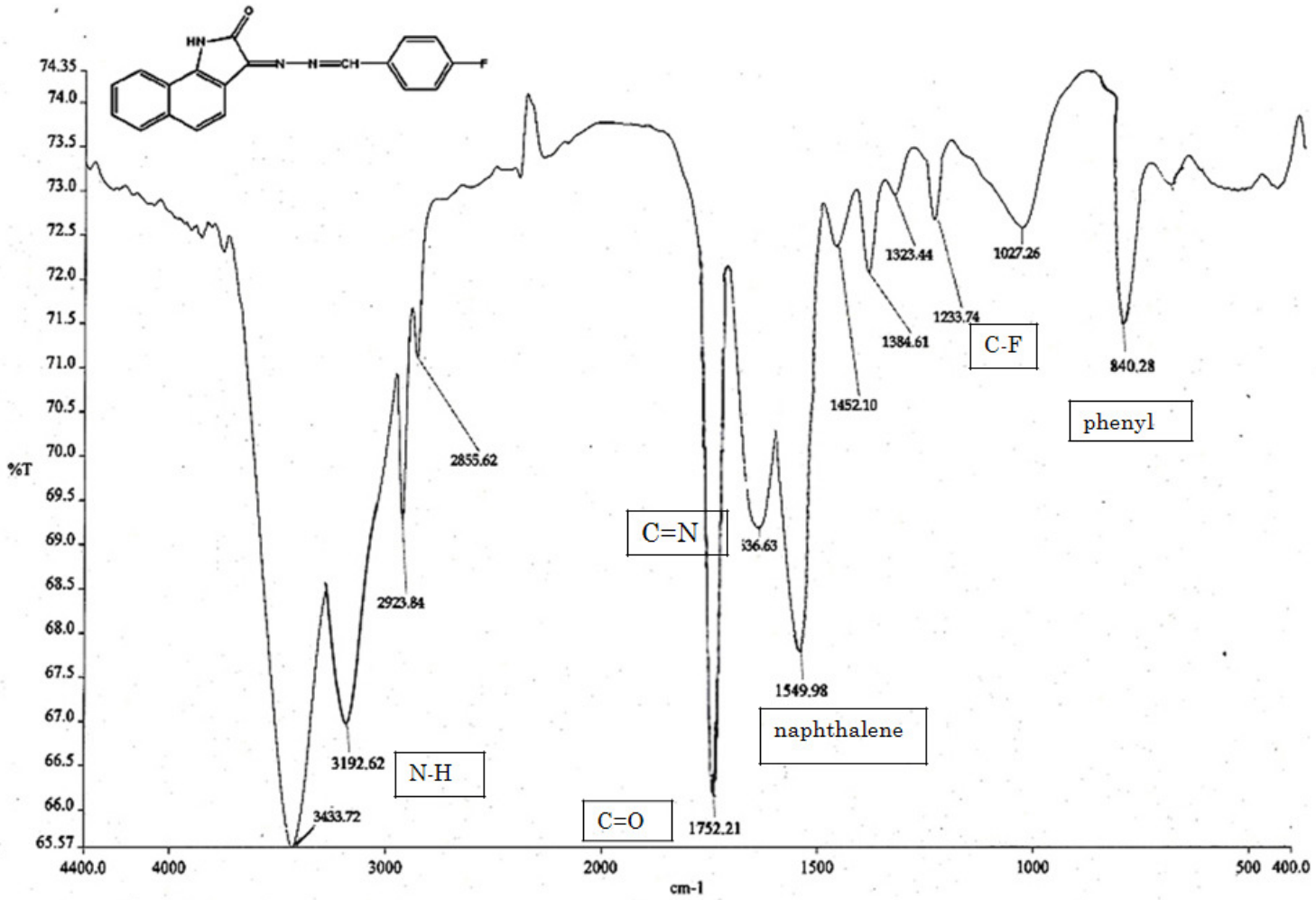


Fig. No. 10. FT-IR Spectrum of Sample Vf

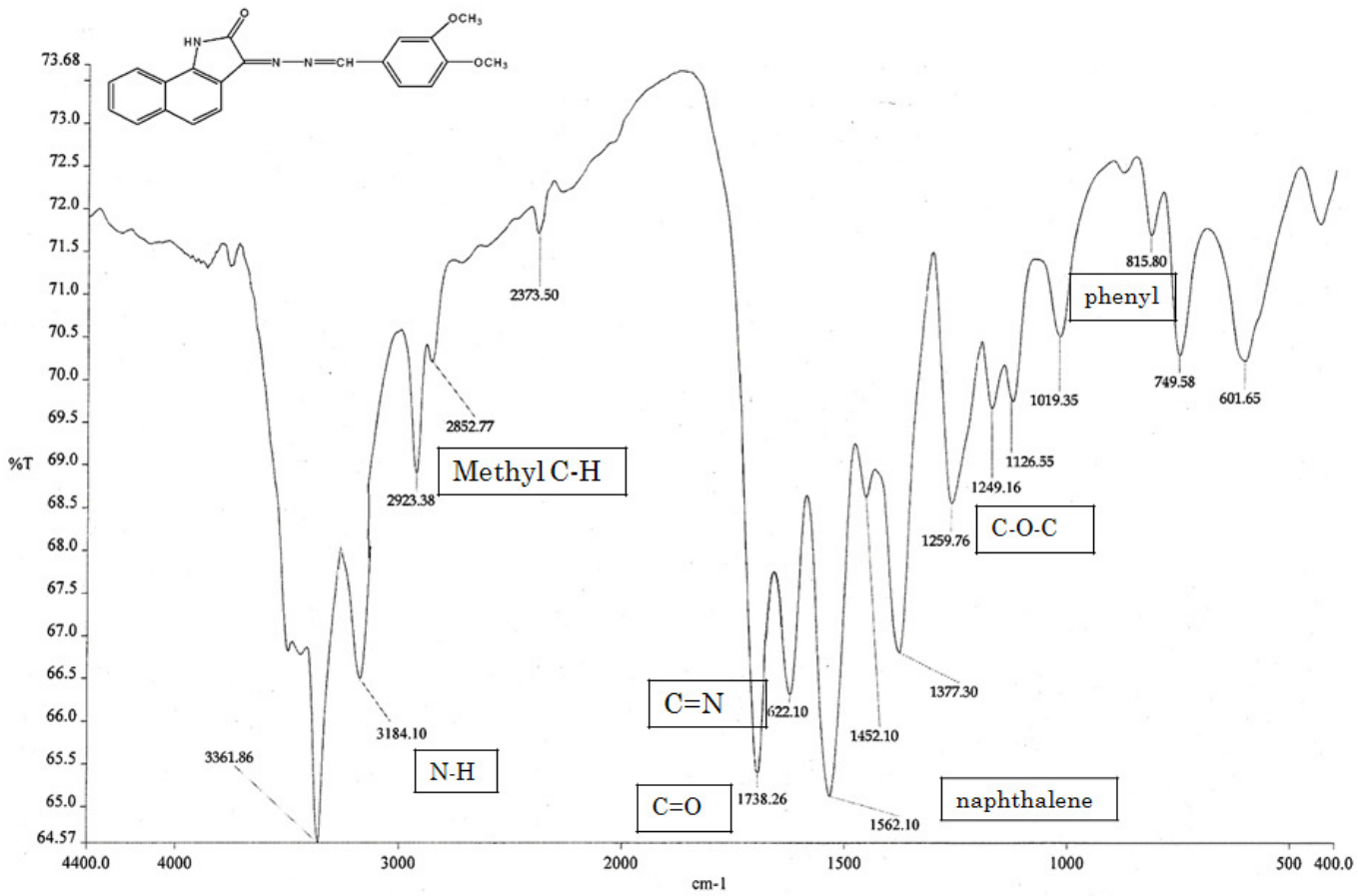


Fig. No. 11. FT-IR Spectrum of Sample Vg

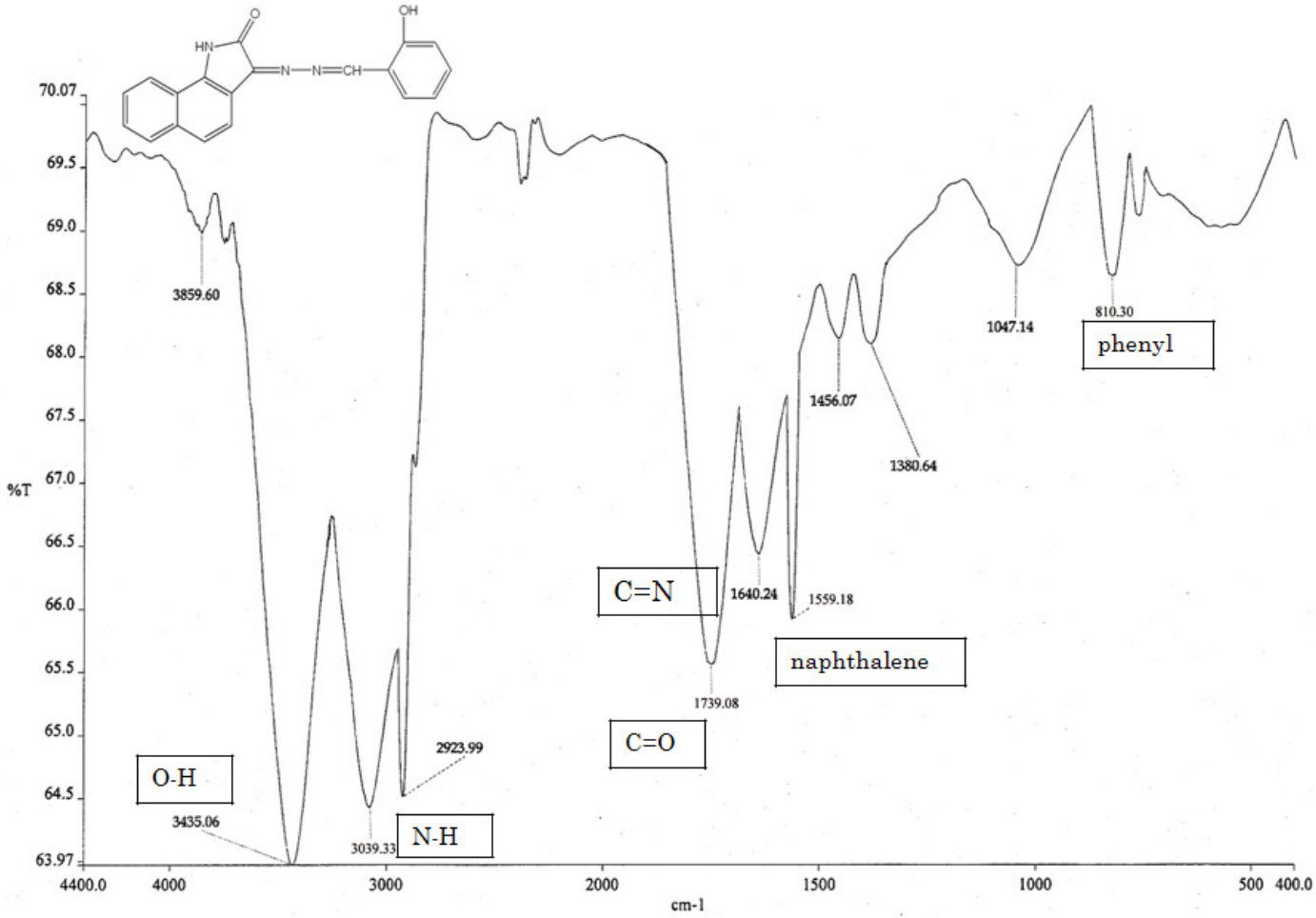


Fig. No. 12. FT-IR Spectrum of Sample Vh

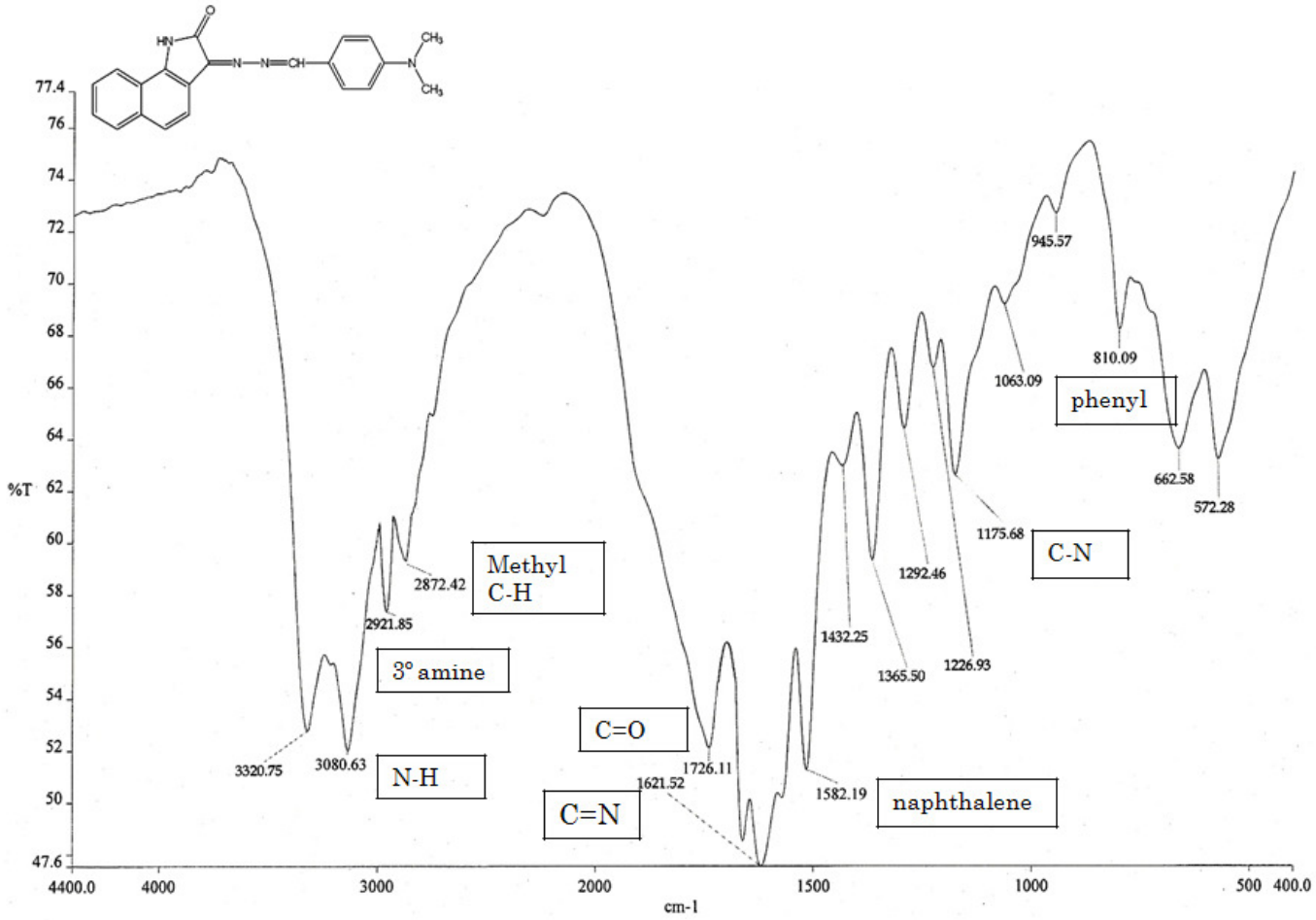


Fig. No. 13. FT-IR Spectrum of Sample Vi

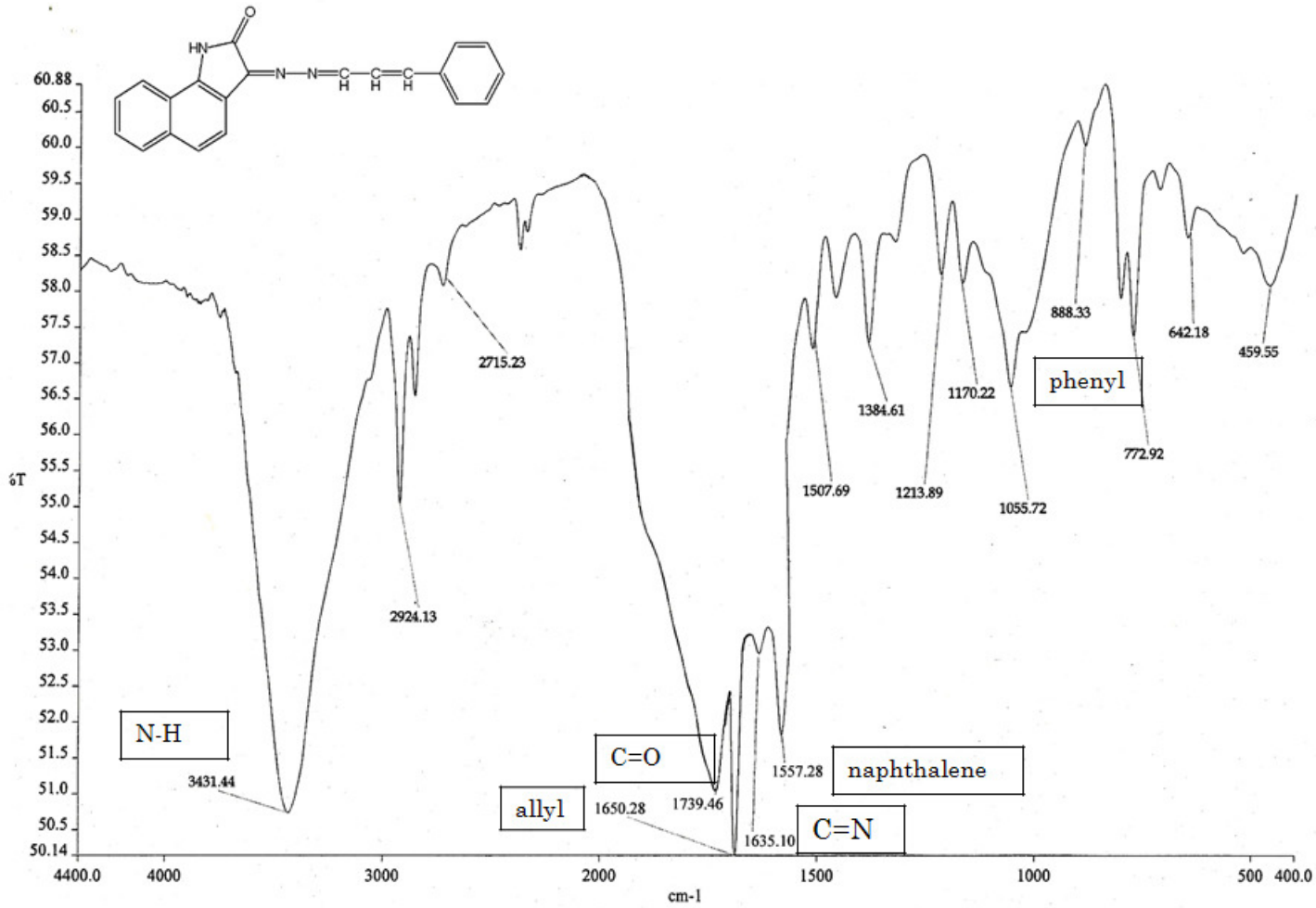


Fig. No. 14. FT-IR Spectrum of Sample Vj

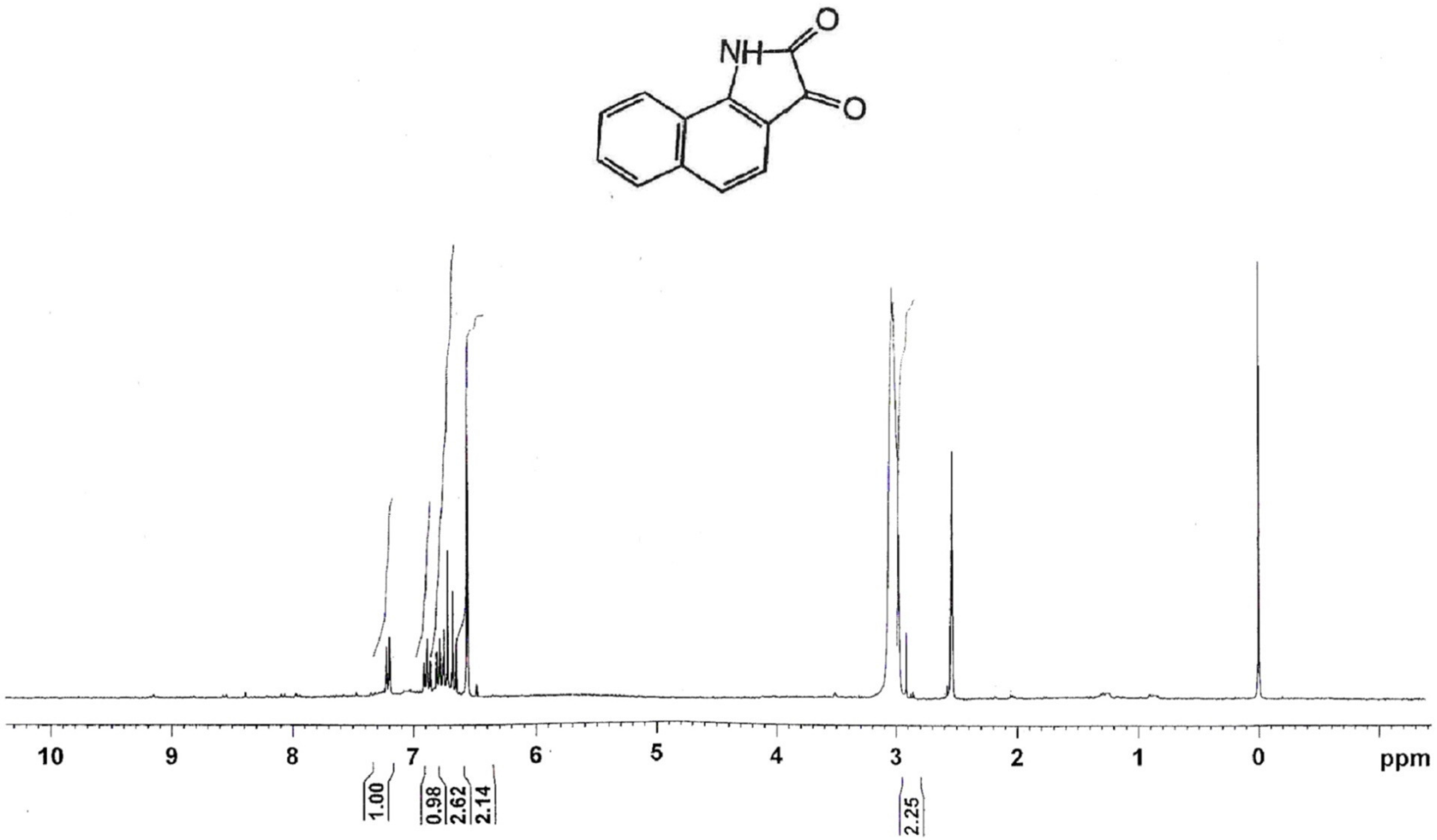


Fig. No. 15 ^{1}H NMR Spectrum of 1H-benzo[g]indole-2,3-dione (III)

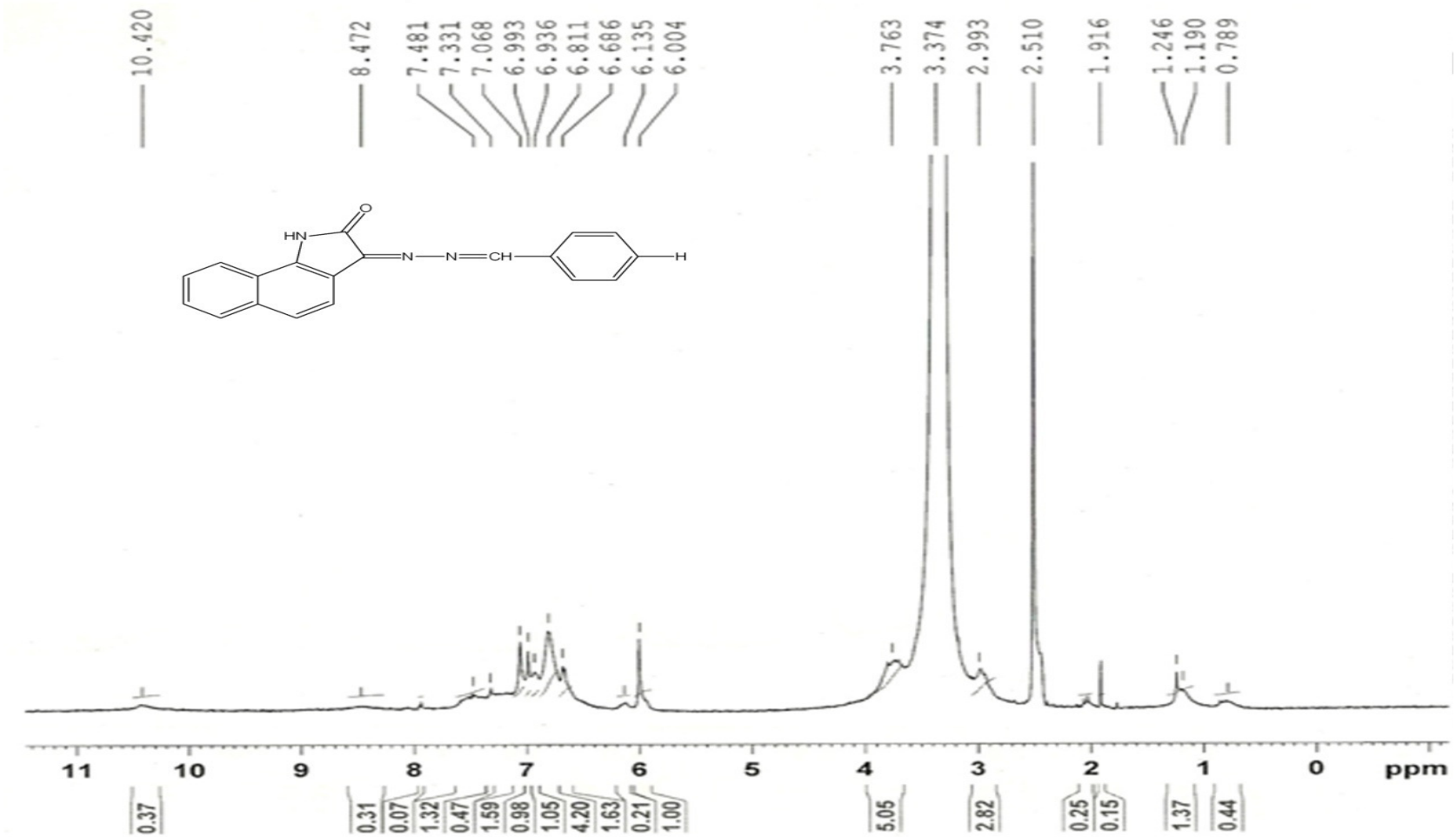


Fig. No. 16. ^{1}H NMR Spectrum of Sample Va

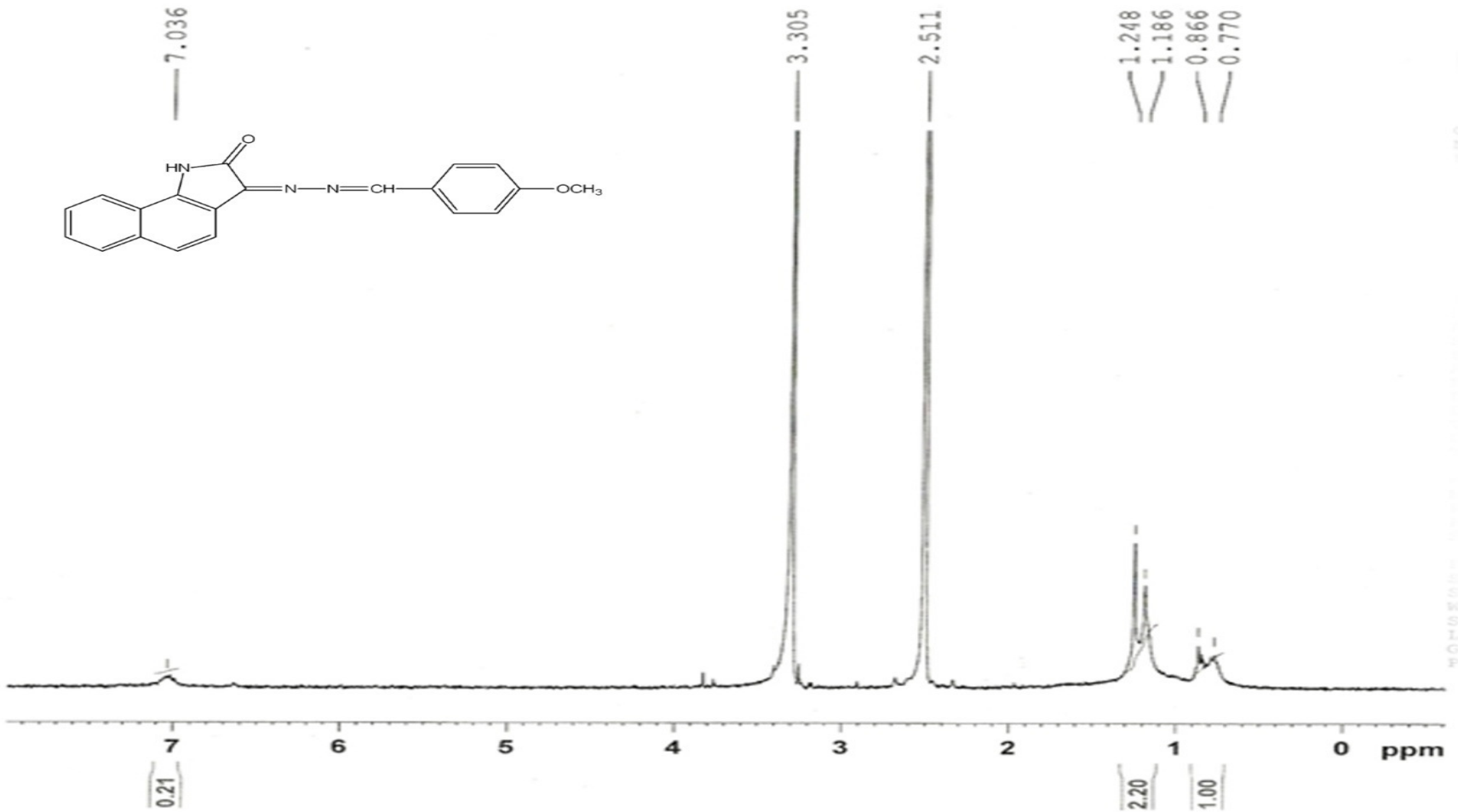


Fig. No. 17. ^{1}H NMR Spectrum of Sample Vb

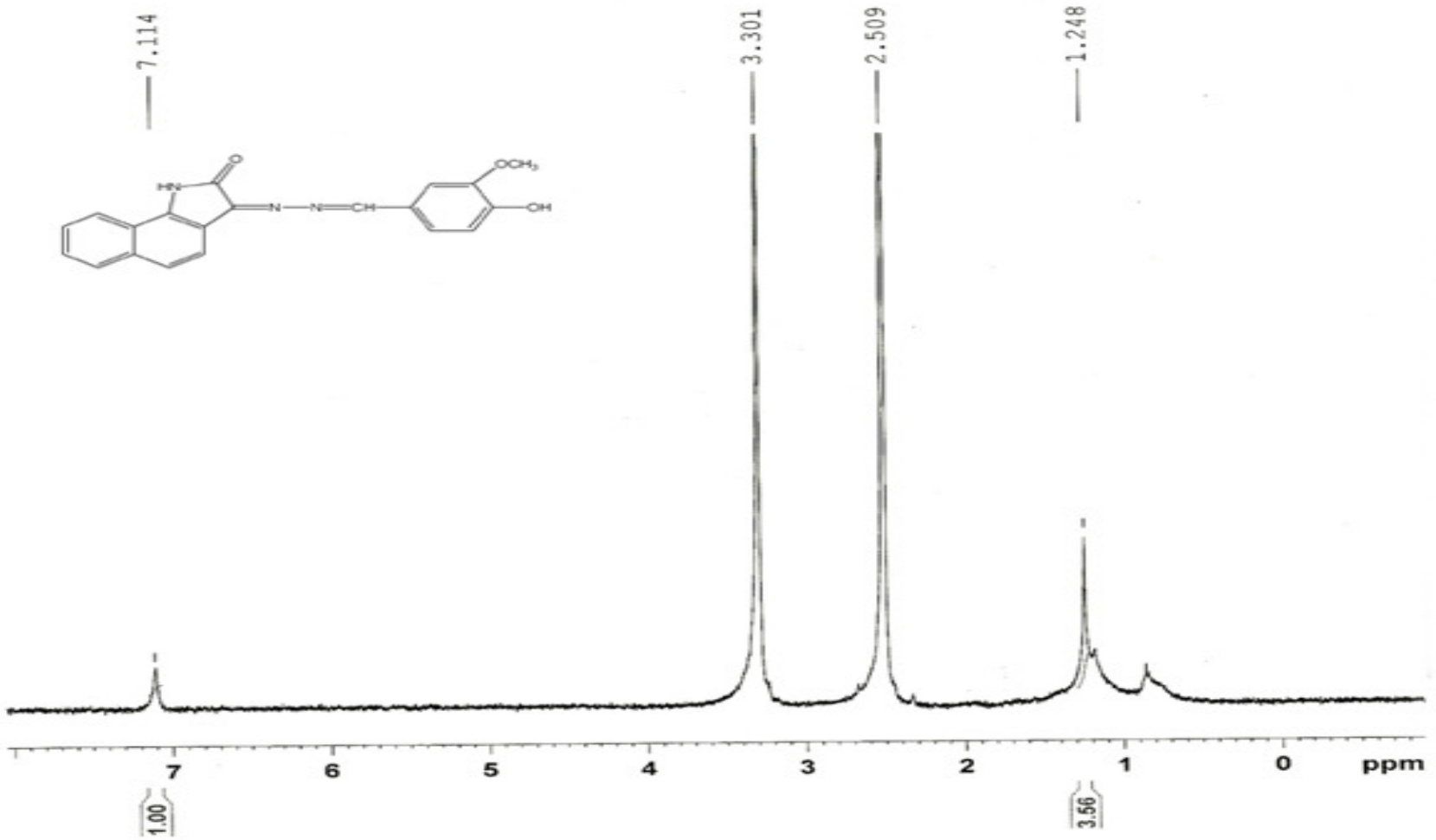


Fig. No. 18. ^{1}H NMR Spectrum of Sample Vc

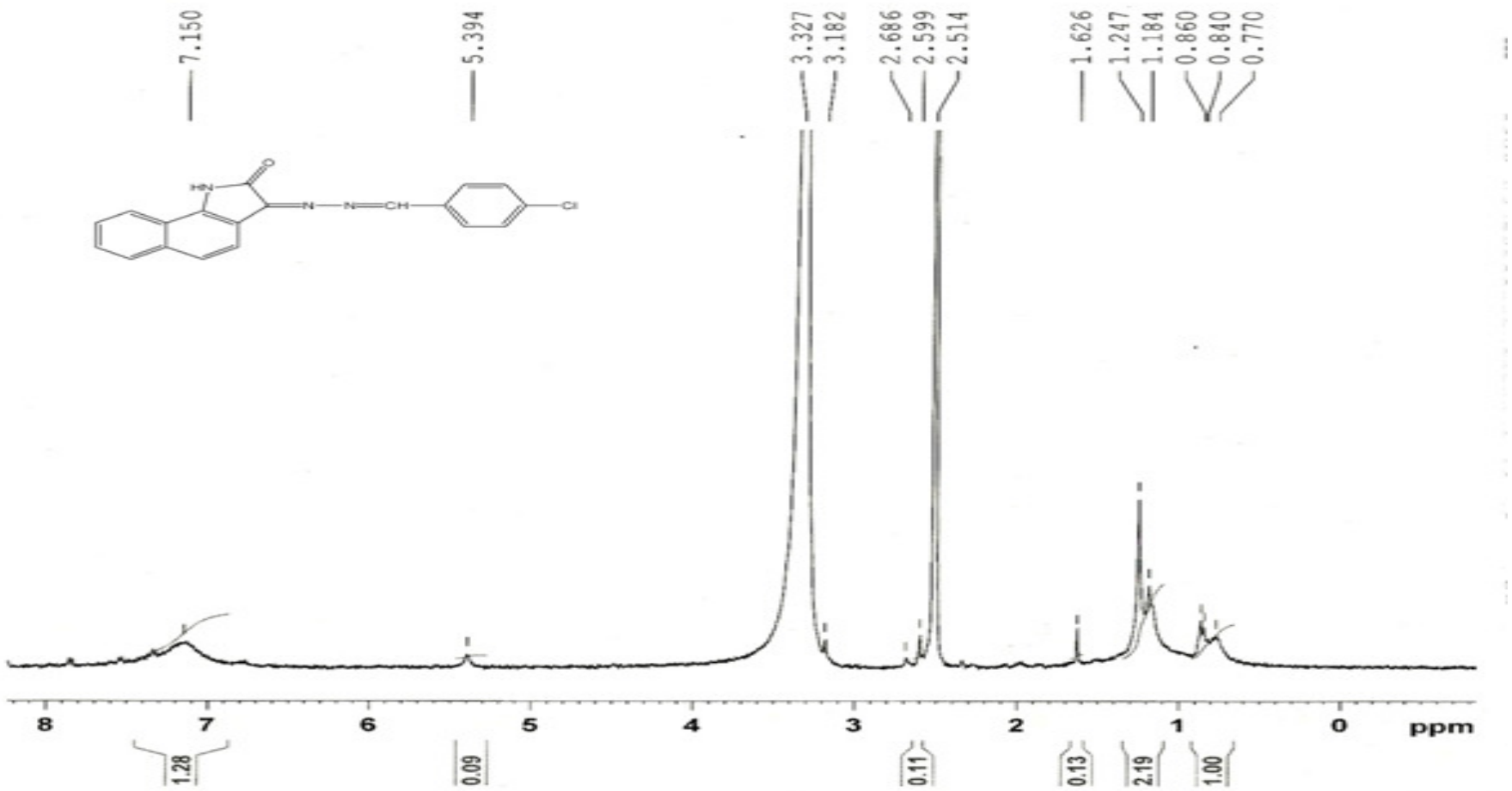


Fig. No. 19. ^{1}H NMR Spectrum of Sample Vd

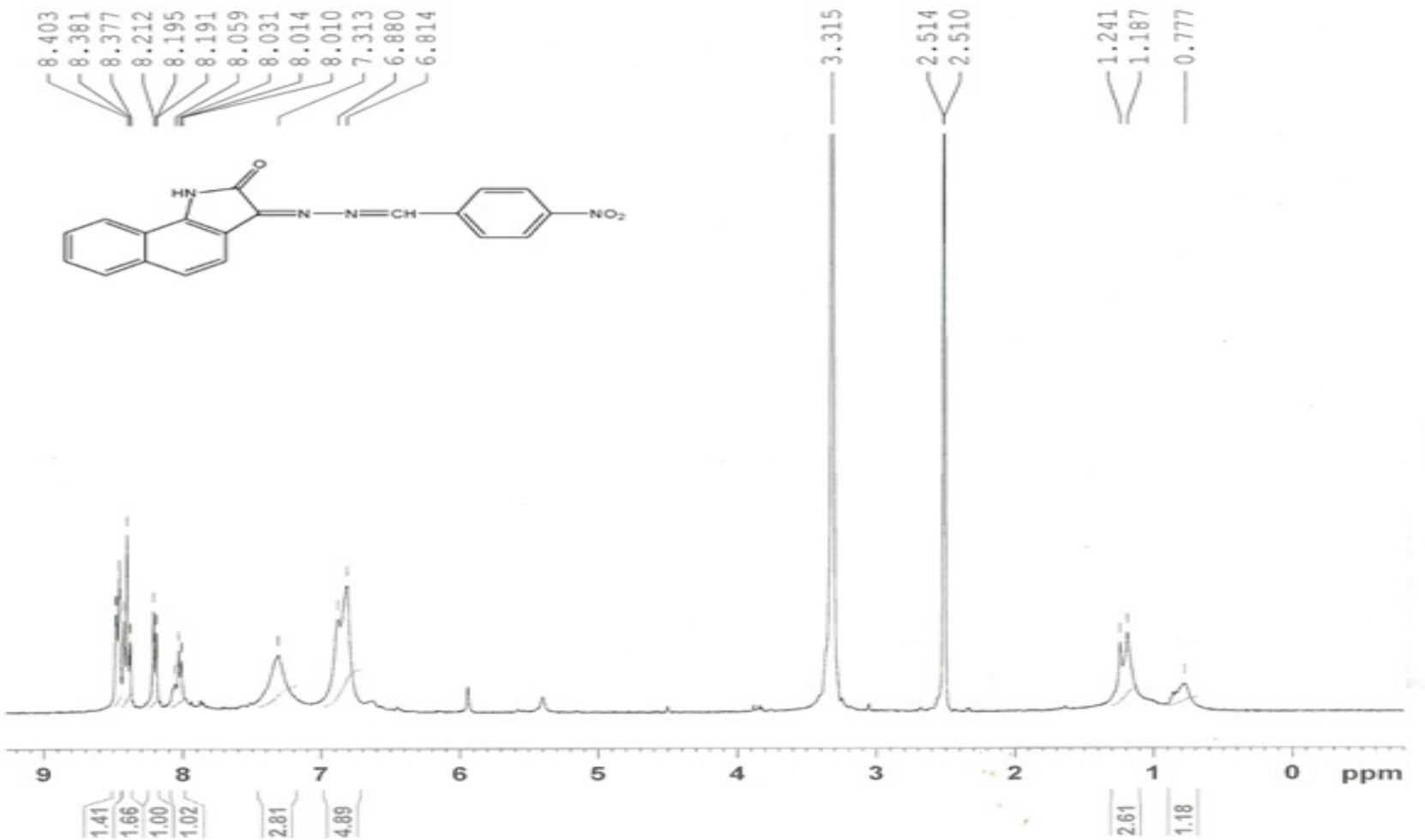


Fig. No. 20. ^{1}H NMR Spectrum of Sample Ve

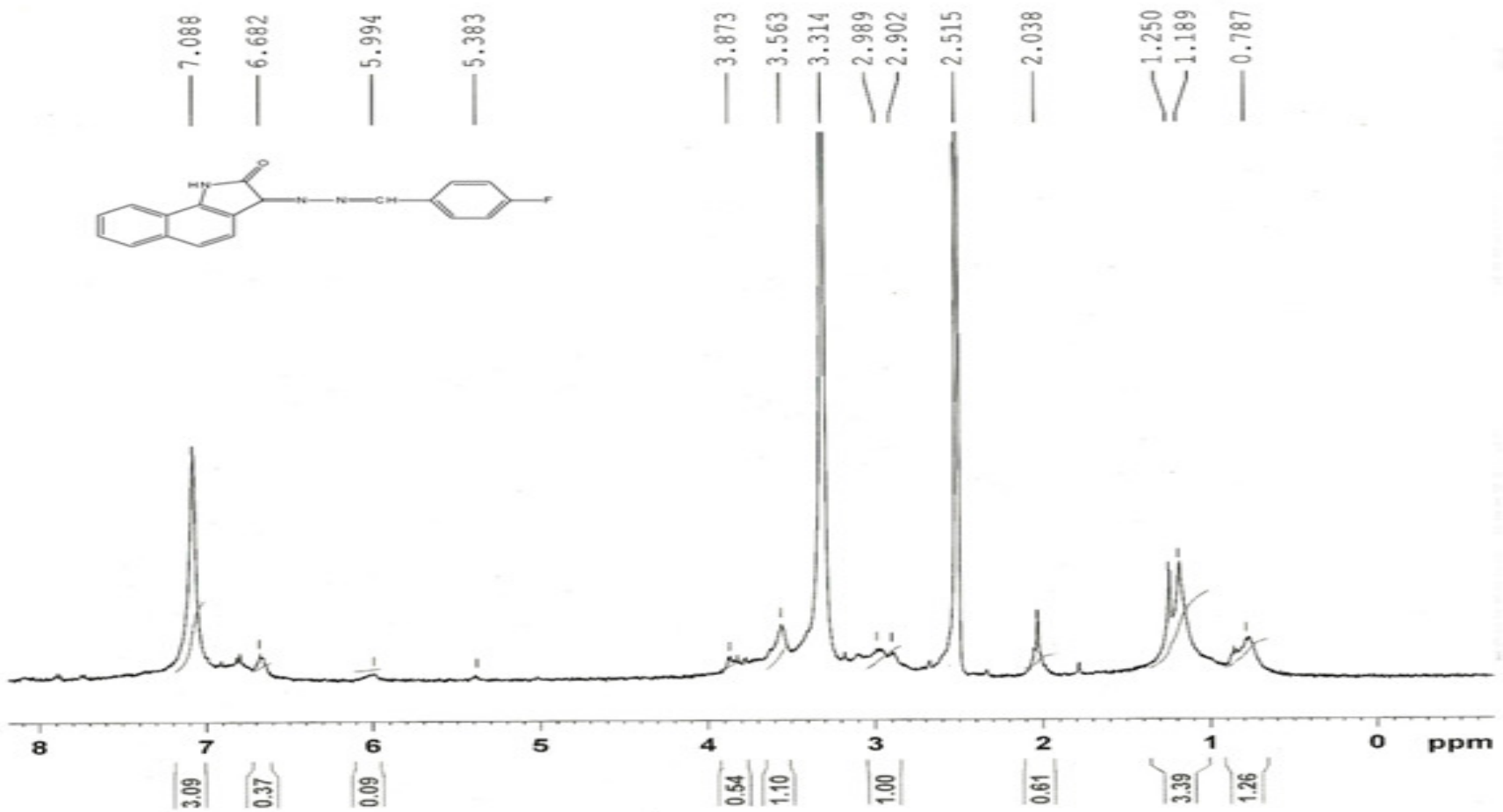


Fig. No. 21. ^{1}H NMR Spectrum of Sample Vf

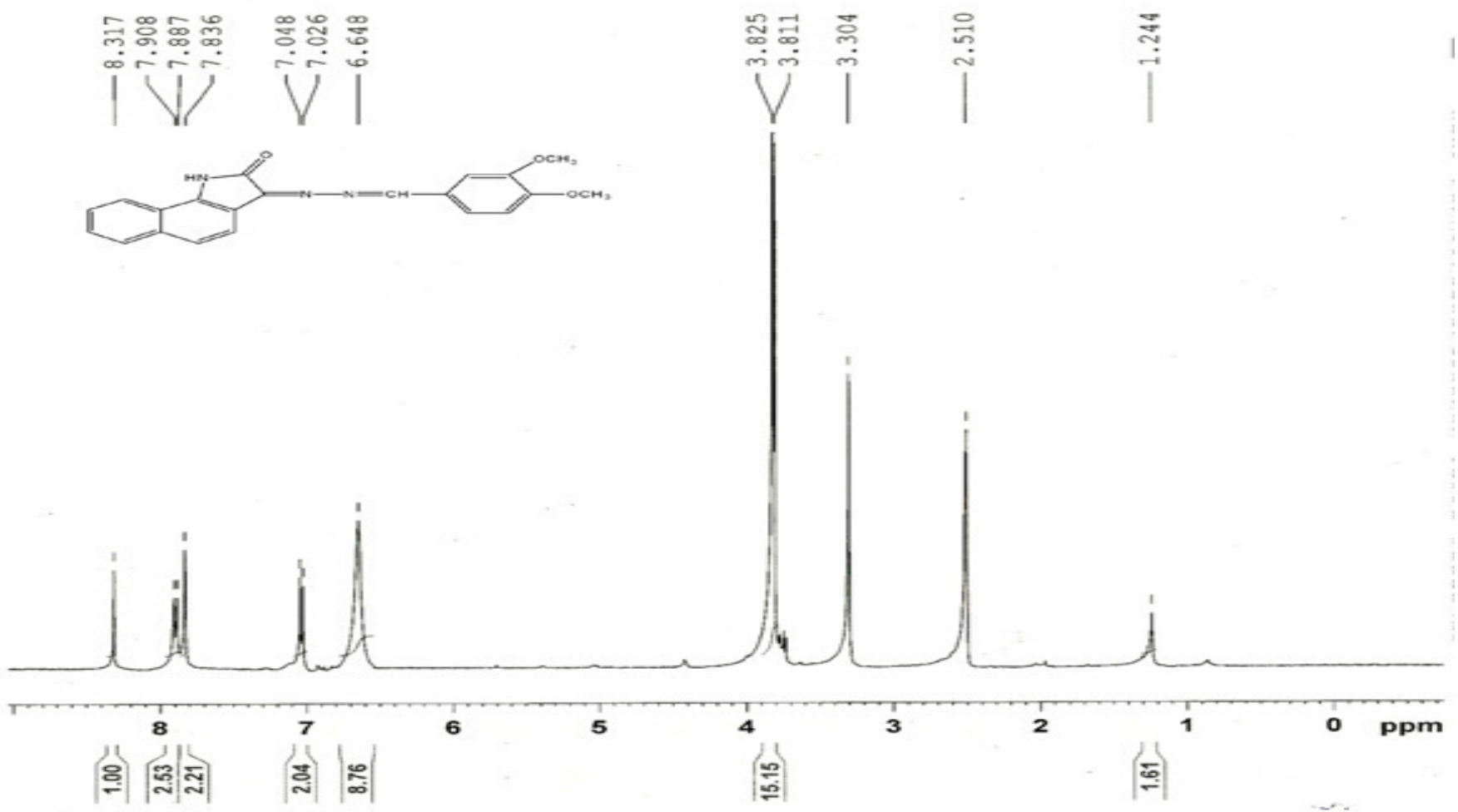


Fig. No. 22. ^{1}H NMR Spectrum of Sample Vg

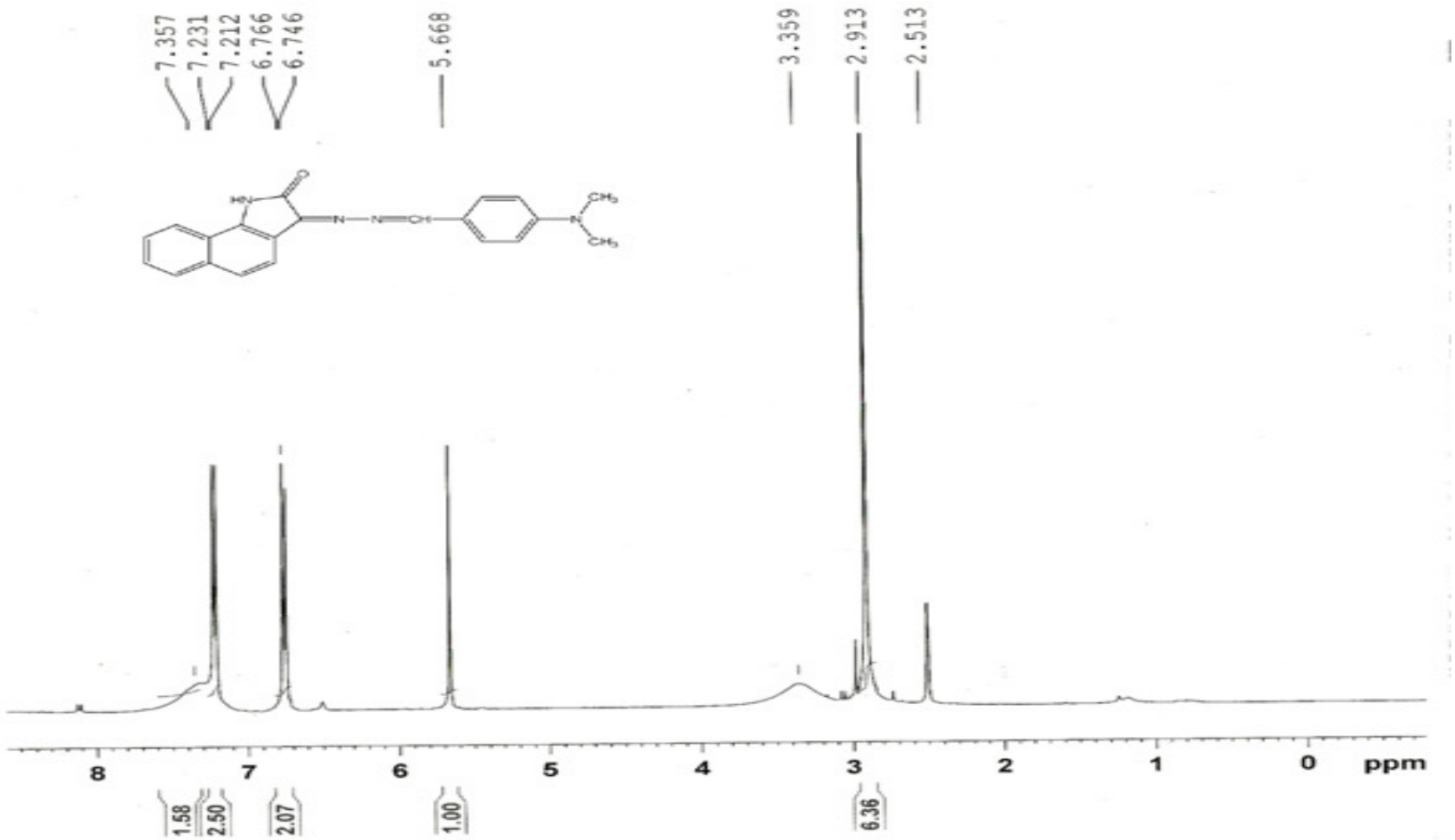


Fig. No. 23. ^{1}H NMR Spectrum of Sample Vi

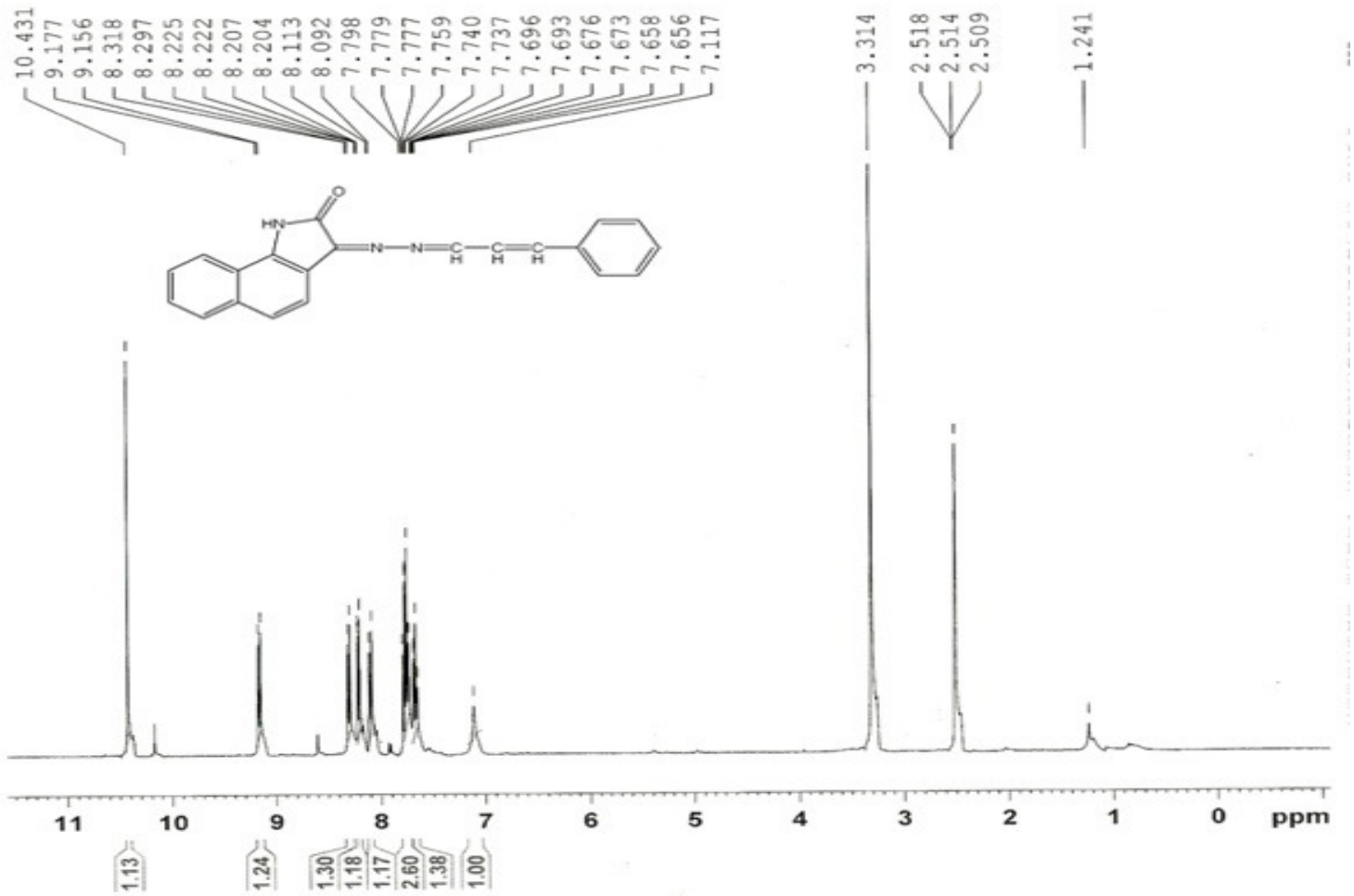


Fig. No. 24. ^{1}H NMR Spectrum of Sample Vj

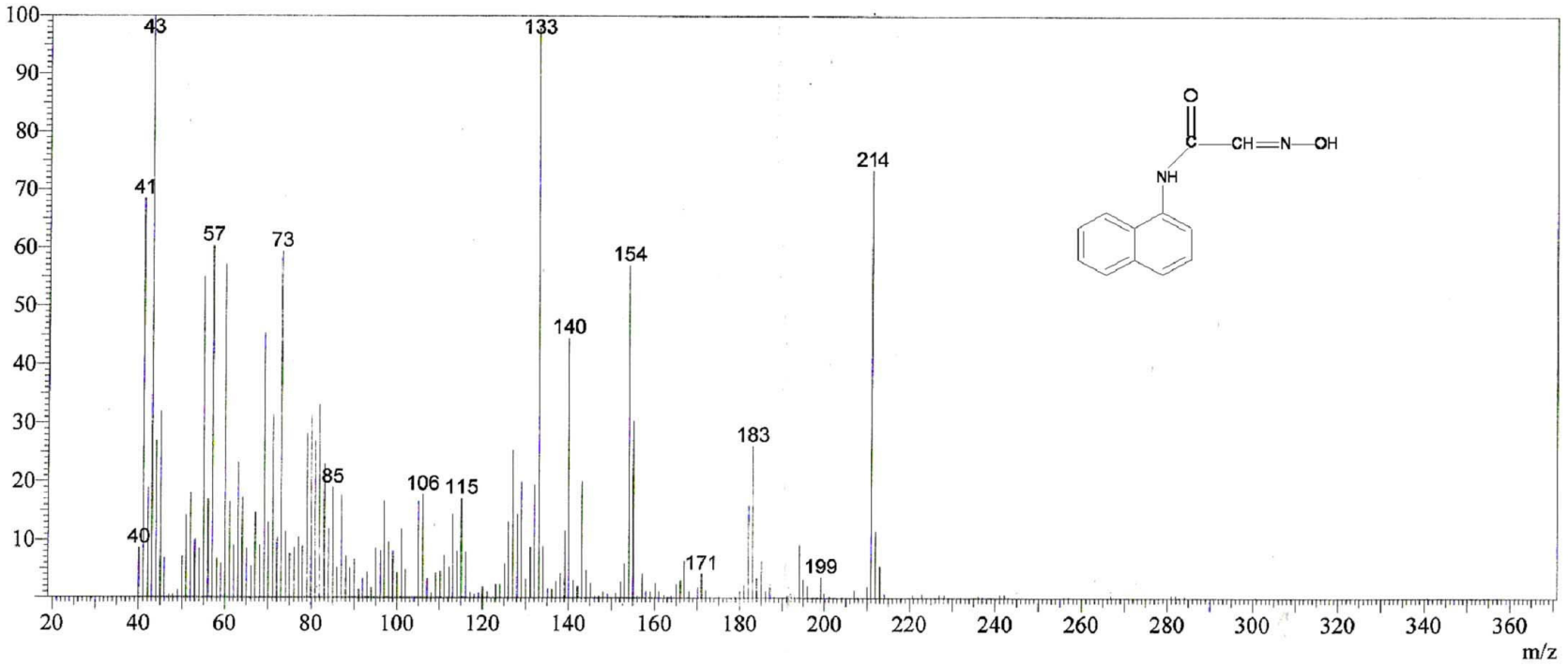


Fig. No. 25 Mass Spectrum of (2)-(hydroxyimino)-N-(naphthalen-1-yl)ethanamide (II)

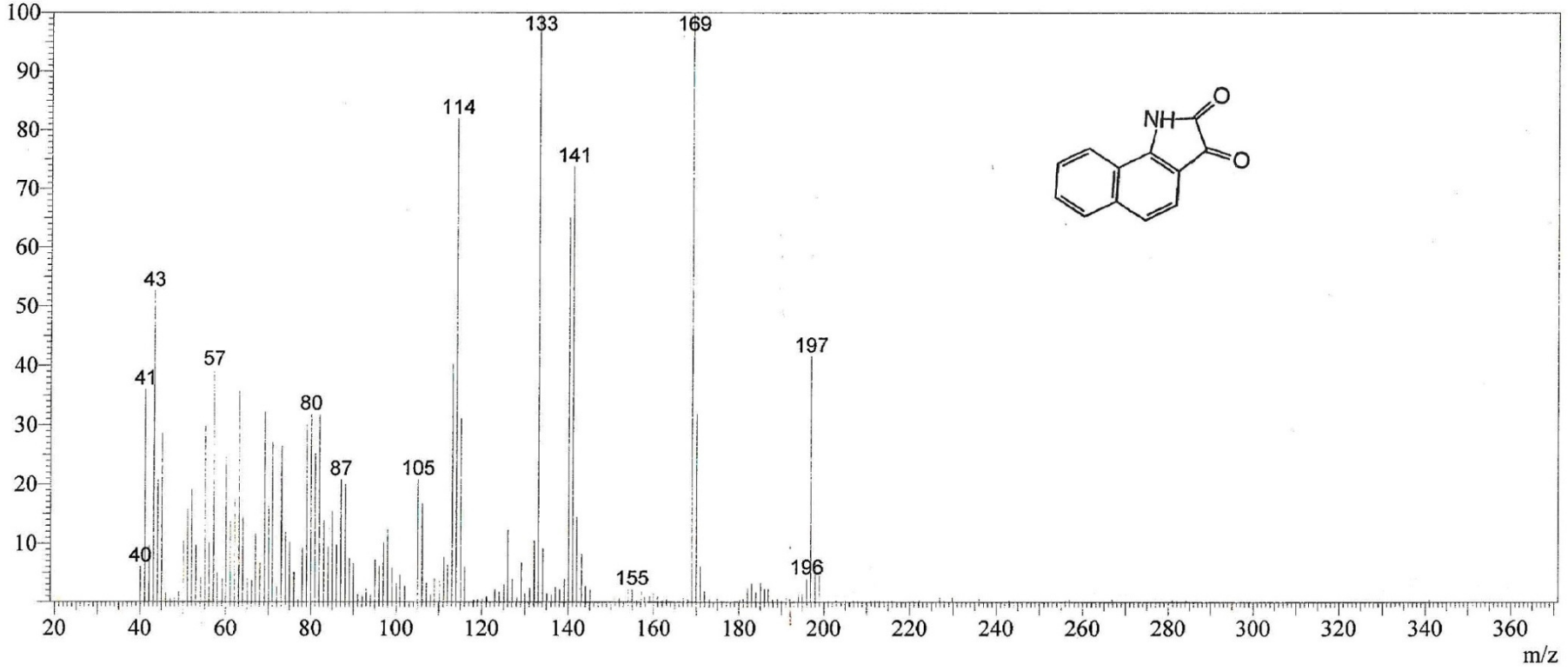


Fig. No. 26 Mass Spectrum of 1H-benzo[g]indole-2, 3-dione (III)

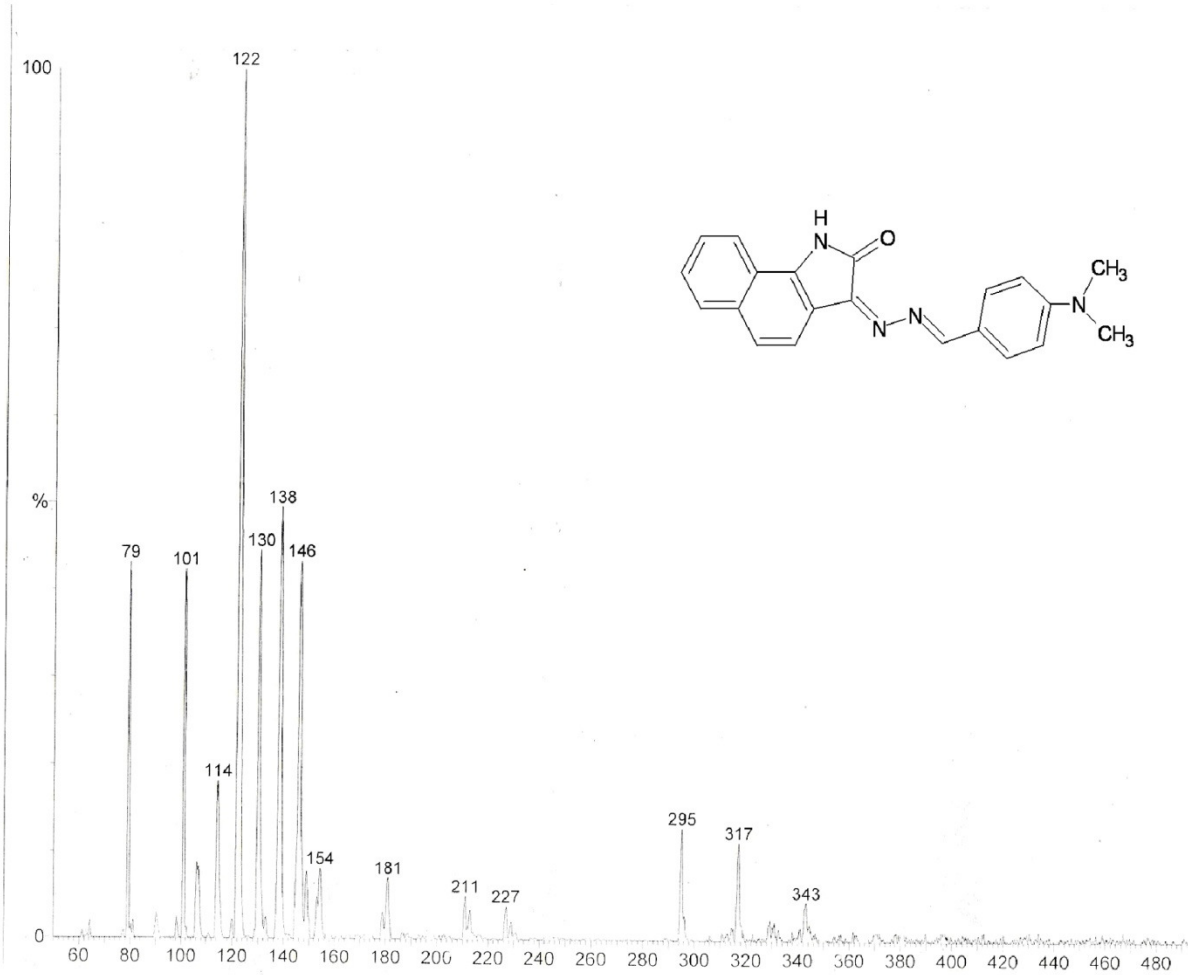


Fig. No. 27 Mass Spectrum of Sample (Vi)

5. BIOLOGICAL SCREENING

5.1 Introduction

Microorganism

A microorganism or microbe is an organism that is microscope (too small to be seen by the naked human eye).A subject that began with Anton van Leeuwenhoek's discovery of microorganism in 1675, using a microscope of his own design.

Microorganisms are incredibly diverse and include bacteria, fungi, archaea, and protests, as well as some microscopic plants and animals. Microbes are also exploited by people in biotechnology, both in traditional food and beverage preparation, as well as modern technologies based on genetic engineering. However, pathogenic microbes are harmful, since they invade and grow within other organisms, causing diseases that kill millions of people, other animals, and plants every year.

Bacteria

Bacteria are unicellular microorganisms. Typically a few micrometers in length, bacteria have a wide range of shapes, ranging from spheres to rods to spirals. Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste, seawater, and deep in the Earth's crust.

Although the vast majority of these bacteria are rendered harmless or beneficial by the protective effects of the immune system, a few are pathogenic bacteria and cause infectious diseases. In developed countries, antibiotics are used to treat bacterial infections and in various agricultural processes, so antibiotic resistance is becoming common.

Pathogenic bacteria

If bacteria form a parasitic association with other organisms, they are classed as pathogens. Pathogenic bacteria are a major cause of human death disease and cause infections such as tetanus, typhoid fever, diphtheria, syphilis, cholera, food borne illness, leprosy and tuberculosis. A pathogenic cause for a known medical disease may only be discovered many years after, as was the case with Helicobacter pylori and peptic ulcer disease.

Each species of pathogen has a characteristic spectrum of interactions with its human hosts. Some organisms such as staphylococcus and streptococcus can cause skin infections, pneumonia, and meningitis, a systemic inflammatory response producing shock, massive vasodilatation and death. Yet these organisms are also part of the normal human flora and usually exist on the skin or in the nose without causing any disease at all. Other organisms invariably cause disease in humans, such as Rickettsia, which are obligate intracellular parasites able to grow and reproduce only within the cells of other organisms. One species of Rickettsia causes typhus, while another causes Rocky Mountain spotted fever. Chlamydia, another phylum of obligate intracellular parasites, contains species that can cause pneumonia, or urinary tract infection and may be involved in coronary heart disease.

Treatment

Bacterial infections may be treated with antibiotics, which are classified as bactericidal if they kill bacteria, or bacteriostatic if they just prevent bacterial growth. There are many types of antibiotics and each class inhibits a process that is different in the pathogen from that found in the host. Examples of how antibiotics produce selective toxicity are chloramphenicol and puromycin, which inhibit the bacterial ribosome, but not the structurally different eukaryotic ribosome. Antibiotics are used both in treating human disease and in intensive farming to promote animal growth, where they may be contributing to the rapid development of antibiotic resistance in bacterial populations. Disinfectants such as bleach are used to kill

bacteria or other pathogens on surfaces to prevent contamination and further reduce the risk of infection.

Antibiotic

An antibiotic is a chemotherapeutic agent that inhibits or abolishes the growth of micro-organisms, such as bacteria, fungi, or protozoa. The term originally referred to any agent with biological activity against living organisms. However, "antibiotic" now is used to refer to substances with anti-bacterial, anti-fungal, or anti-parasitical activity. With advances in organic chemistry many antibiotics are relatively small molecules with a molecular weight less than 2000 Da.

Most antibacterial antibiotics do not have activity against viruses, fungi, or other microbes. Anti-bacterial antibiotics can be categorized based on their target specificity. "narrow-spectrum" antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, while broad spectrum antibiotics affect a wide range of bacteria.

The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the microbe to inactivate or excrete the antibiotic. Some anti-bacterial antibiotics destroy bacteria (bactericidal), whereas others prevent bacteria from multiplying (bacteriostatic).

In the last few years, three new classes of antibiotics have been brought in to clinical use. This fallows a 40-year hiatus in discovering new classes of antibiotic compounds.

Classification of antibiotics

Eg: Amikacin, Streptomycin, neomycin, gentamycin.

Cephalosporins

First generation Eg: cefadroxil, cefazolin.

Second generation Eg: cefuroxime, cefmandole.

Third generation Eg: cefixime, cefotoxime.

Fourth generation Eg: cefepime.

Glycopeptides

Eg: vancomycin

Macrolides

Eg: erythromycin, clarythromycin

Penicillins

Eg: ampicillin, amoxicillin.

Sulphonamides

Eg: sulphamethazole, sulfasalazine.

Quinolones

Eg: ciprofloxacin, norfloxacin.

Tetracyclines

Eg: tetracycline, doxycycline.

Others

Eg: clindamycin, vancomycin.

Fungus

The fungi are heterotrophic organisms characterized by a chitinous cell wall, and in the majority of species, filamentous growth as multi cellular hyphae forming a mycelium; some fungal species also grow as single cells. The fungi are a monophyletic group that is phylogenetically clearly distinct from the morphologically similar slim molds (myxomycetes) and water molds (oomycetes).

Many fungal species have long been used as a direct source of food, such as mushrooms and truffles and in fermentation of various food products, such as wine, beer, and soy sauce. More recently, fungi are being used as sources for antibiotics and various enzymes, such as cellulases, pectinases, and proteases, important for industrial use or as active ingredients of detergents. Several species of the fungi are significant pathogens of humans and other animals, and losses due to diseases of crops or food spoilage caused by fungi can have a large impact on human food supply and local economies.

As pathogens and parasites

However many fungi are parasites on plants, animals, and other fungi. Serious fungal pathogens of many cultivated plants causing extensive damage and losses to agriculture and forestry include the rice blast fungus Magnaporthe oryzae, tree pathogens such as Ophiostoma ulmi and Ophiostoma novo-ulmi causing Dutch elm disease, and Cryphonectria parasitica responsible for chestnut blight, and plant-pathogenic fungi in the genera Fuserium, Ustilago, Alternaria, and Cochliobolous; fungi with the potential to cause serious human diseases, especially in persons with immune-deficiencies, are in the genera Aspergillus, Candida, Cryptococcus, Histoplasma, and Pneumocystis. Several pathogenic fungi are also responsible for relatively minor human diseases, such as athlete's foot and ringworm. Some fungi

are predators of nematodes, which they capture using an array of devices such as constricting rings or adhesive nets.

Treatment

An **antifungal drug** is medication is used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others.

Classification of antifungals

Polyenes

Eg: Nystatin, Amphotericine B, and Candicin.

Imidazoles

Eg: Miconazole, Ketoconazole, Clotrimazole.

Triazoles

Eg: Fluconazole, Itraconazole, Isavuconazole.

Allylamines

Eg: Terbinafine, Amorolfine, Naftifine.

Echinocandins

Eg: Anidulafungin, Capsofungin, Micafungin.

Others

Eg: Ciclopirox, Flucytosine, Griseofulvin.

Antimicrobial

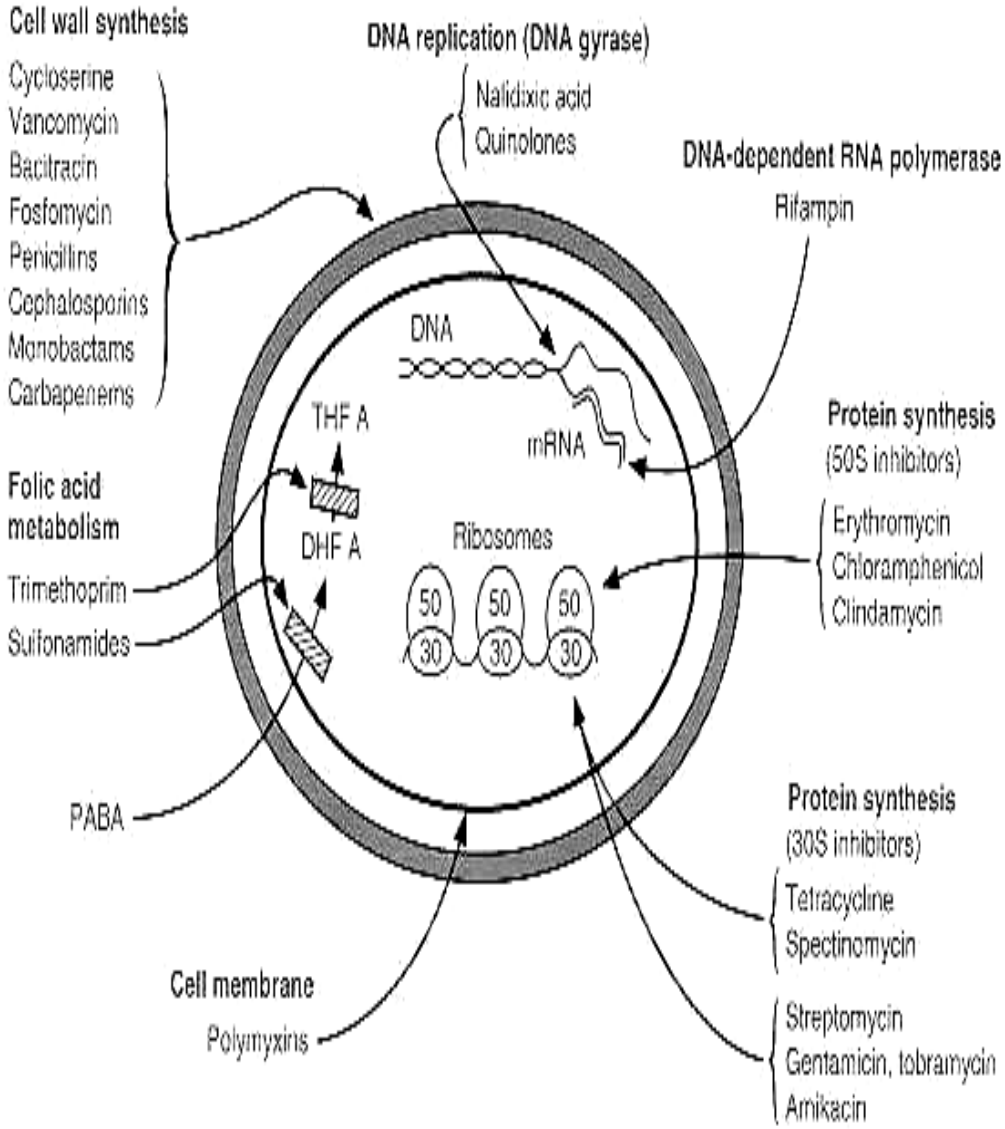
An **antimicrobial** is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, or viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic).

However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the sickest patients-those in the hospital. Currently bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found, thus future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means (antibacterial activity), fungi (antifungal activity), viruses (antiviral activity), or parasites (antiparasitic activity).

History of antibacterial drug introductions and approval

Introduced year	**Class of drug**
1935	Sulfonamides
1941	β-lactams(Penicillin)
1944	Chloramphenicol
1950	Tetracyclines
1952	Macrolides/ Lincosamides/ streptogramins
1956	Glycopeptides
1957	Rifamycins
1959	Nitromidazoles
1962	Quinolones Kefauver-Harris Amendments 1962
1968	Trimethoprim
2000	Oxazolidinones
2003	Lipopeptides

Fig. No. 28 Mechanism of action of antimicrobial agents[88]



All antimicrobial agents act in five different modes:

1. The inhibition of cell wall synthesis:

The cell wall of the bacterium consists of macromolecular network called Peptidoglycon. Peptidoglycon is found only in bacterial cell wall. Penicillin and certain other antibiotics prevent the synthesis of intact peptidoglycon consequently the cell wall wills greatly weakend in its integrity and the cell wall undergoes lysis leading to cell death.

2. Inhibition of protein synthesis:

Protein synthesis is a common feature of both prokaryotic and eukaryotic organism. A significant difference in the machinery of protein synthesis, the structure of ribosomes. The difference in ribosomal structure accounts for the selectivity toxicity of antibiotics that affect protein synthesis. The antibiotics like Chloramphenicol, Erythromycin, Streptomycin and Tetracyclines act on the protein synthesis process.

3. Injury to plasma membrane:

Certain antibiotics like polypeptide antibiotics bring about changes in the permeability of the plasma membrane. These changes result in the loss of important metabolites from the microbial cell. Polymixin B causes disruption of the plasma membrane by attaching to the phospholipids of the membrane.

4. The inhibition of nucleic acid synthesis:

A number of antibiotics interfere with the process of DNA replication and transcription in microorganisms. Some drugs with this mode of action have extremely limited usefulness because they interfere with mammalian DNA and RNA as well. Rifampicin and Quinolones are widely used in chemotherapy because they are more selectively toxic.

5. Inhibition of synthesis of essential metabolites:

A particular enzymatic activity of a microorganism can be competitively inhibited by a substance (anti metabolite) that closely resembles the normal substrate for the enzyme. An example of competitive inhibition is the relationship between the anti metabolite sulfonamide and p-amino benzoic acid (PABA).

In view of varied biological and pharmacological importance of different Isatins, it has been prompted us to evaluate the new series of 3-[(2) - (phenyl methylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-ones (Va-j) for antimicrobial and antifungal activity.

MATERIALS AND METHODS

Four bacterial test organisms such as ***Bacillus subtilis*** (MTCC 441), ***Staphylococcus aureus*** (MTCC 96), ***Escherichia coli*** (MTCC 722), ***and Proteus vulgaris*** (MTCC 109) were selected and obtained from the Institute Of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were sub cultured in Petri dishes prior to testing. The media used was nutrient agar, nutrient broth procured from Himedia Laboratories, Mumbai. Stock solutions of the synthesized compounds were prepared in the different concentrations, viz.,50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml using dimethylsulfoxide (DMSO) as solvent for antibacterial activity.

5.2 ANTIBACTERIAL ACTIVITY

The antibacterial activity of title compounds was assayed against four different strains of bacteria by agar diffusion method.

Two Gram-Positive Bacteria: ***Bacillus subtilis*** and ***Staphylococcus aureus***

Two Gram-Negative Bacteria: ***Escherichia coli*** and ***Proteus vulgaris***

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial

inhibition can be measured my two methods: one is serial dilution method and the other is diffusion method. The serial dilution method is very useful for the determination of antimicrobial activity. It is not much useful for the quantitative detection tests and also for the evaluation of large number of compounds. The agar diffusion is of three types.

1. Cup-plate method (disc method)
2. Filter-paper strip method
3. Gradient plate method

The method adopted in this investigation was cup-plate method. In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds were introduced into the discs and the diameter of zone of inhibition was measured.

CULTURED MEDIUM

Nutrient broth was used for the preparation of inoculums of the bacteria and the nutrient agar used for the screening method.

Composition of Medium, nutrient agar:

Peptone	5.0gm
Sodium chloride	5.0gm
Beef extract	1.5gm
Yeast extract	1.5gm
Agar	1.5gm
Distilled water	1000ml
PH	7.4 ± 0.2

The test organism was sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37 ± 1ºC for 24 h, they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of stock culture to nutrient broth. The flasks were incubated at 37 ± 1ºC for 48 h before the experimentation.

Solution of test compounds were prepared by dissolving 10 mg each in dimethylsulfoxide (DMSO,10 ml).A reference standard for Gram-positive and Gram-negative bacteria was made by dissolving accurately weighed quantities of Ampicillin in DMSO (10 µg/ml).

The nutrient agar medium was sterilized by autoclaving at 121ºC (15 lb/sq.inch) for 15 min. Petri-plates, tubes and flasks plugged in cotton were sterilized in hot-air oven at 160ºC for an hour. Into each sterilized Petri-plate (10 cm diameter), about 27 ml of molten nutrient agar medium inoculated with the respective strain of bacteria (50 µl of inoculum into each plate) was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, three discs of 6 mm diameter were made with a sterile borer. These solutions at concentrations (200 µg/ml, 150 µg/ml, 100 µg/ml) was added to respective disc aseptically and labeled accordingly. The plates were kept undisturbed for 1 hour at room temperature to allow the diffusion of the solution properly in the nutrient agar medium. After incubation of the plates at 37 ± 1ºC for 24 h, the diameter of zone inhibition surrounding each of discs was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of DMSO to observe the solvent effects and the results were represented in Table-4.

5.3 ANTIFUNGAL ACTIVITY

For the antifungal screening of synthesized compounds, ***Candida albicans and Yeast*** were used.

Sabourad dextrose agar medium (SDA)

Mycological peptone	-	10 gm
Dextrose	-	14 gm
Agar	-	17 gm

The test organisms were sub cultured using SDA medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25ºC for 48 h, were stored at 4ºC in refrigerator. The inoculum was prepared by taking a loopful of stock culture to about 5 ml of sabourad dextrose broth in a test tube. The tubes were incubated at 25ºC for 48-72 h before use.

The solution of test compound was prepared by a similar procedure described under the antibacterial activity. A reference standard solution of Clotrimazole (10 µg/ml) was prepared by dissolving 10 mg of Clotrimazole in 10 ml of dimethylsulfoxide (DMSO).

The SDA medium was sterilized by autoclaving at 121ºC (15 lb/sq.inch) for 15 min. the Petri-plates were sterilized in hot-air oven at 160º C for an hour. Into each sterilized Petri-plate about 27 ml of molten SDA medium was added. Incubated at 30º C for 2 days. After 2 days of incubation, the medium free of contaminations was spreaded with 50 µl of 48 h culturing. After solidification of the cups of 6 mm diameter were made in each plate with sterile borer. Accurately 50 µl of 200 µg, 150 µg, and 100 µg concentrations of test solution was transferred to the respective Petri-plates aseptically and labeled accordingly. The reference standard 50µl was also added to the discs in each plate.

The plates were kept in refrigerator for one hour to allow the solution to diffuse properly into the SDA medium. Then the plates were incubated at 26º C for 72 h at inverted position. The diameter of zone of inhibition was read with help of an antibiotic zone reader. The experiment was performed in triplicate and the results were represented in Table-5.

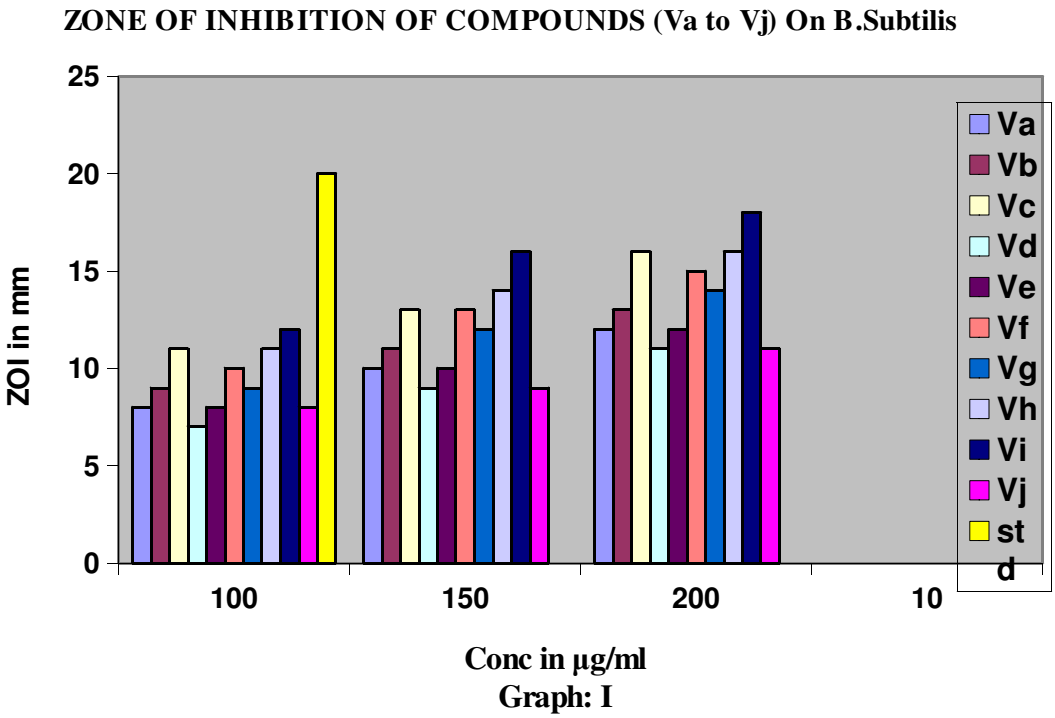
ANTIBACTERIAL ACTIVITY

Table 4: Antibacterial activity of 3-[(2-phenyl methylidene) hydrazono]-1, 3-dihydro-2H-benzo[*g*]indole-2-one derivatives.

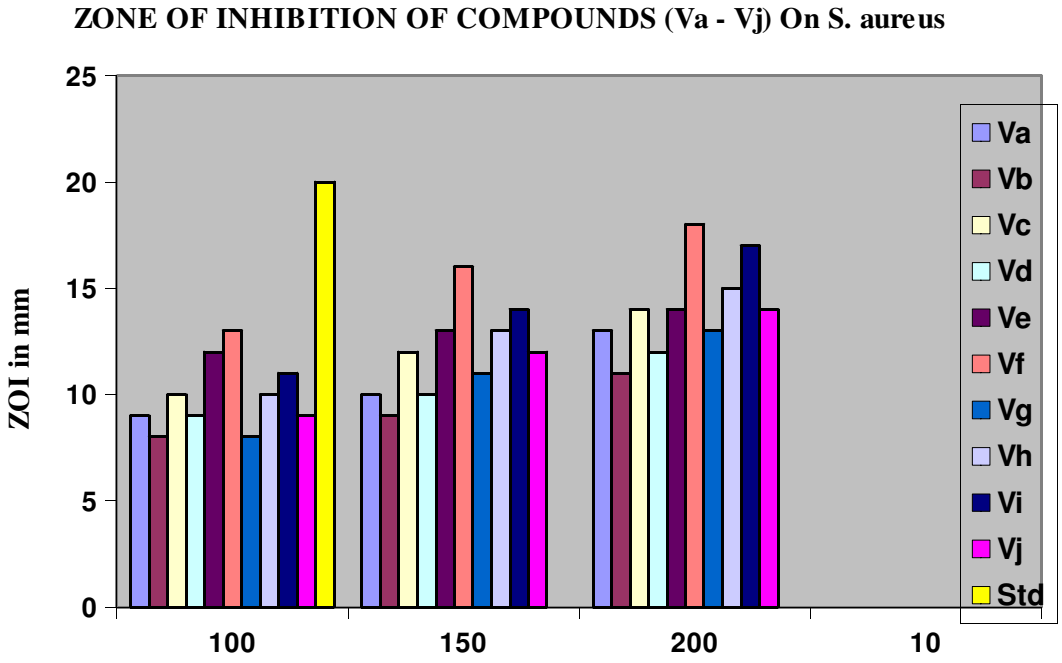
S. No.	Substituents		Conc.	Zone of Inhibition (in mm)			
	Compds	R	Conc. (µg/ml)	B.subtilis	S.aureus	E.coli	P.vulgaris
1	Va	H	100	8	9	8	9
			150	10	12	11	10
			200	13	13	11	12
2	Vb	4-OCH_3	100	9	8	7	8
			150	11	9	9	9
			200	12	11	10	11
3	Vc	4-OH, 3-OCH_3	100	11	10	9	9
			150	13	12	12	11
			200	16	14	14	13
4	Vd	4-Cl	100	7	9	8	10
			150	9	10	10	12
			200	11	12	11	14

5	Ve	4-NO_2	100	8	12	9	12
			150	16	13	11	14
			200	12	14	12	15
6	Vf	4-F	100	10	13	12	10
			150	13	16	13	13
			200	15	18	14	14
7	Vg	3-OCH_3, 4-OCH_3	100	9	8	9	9
			150	12	11	11	12
			200	14	13	12	13
8	Vh	2-OH	100	11	10	13	11
			150	14	13	14	14
			200	16	15	15	15
9	Vi	4-$N(CH_3)_2$	100	12	11	11	13
			150	14	14	12	15
			200	18	17	14	17

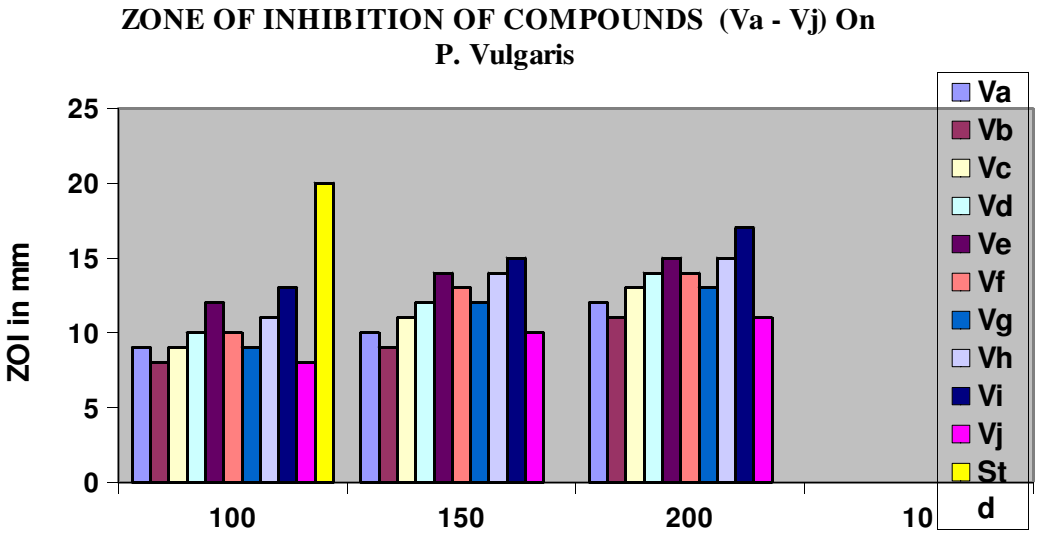
10	Vj	1-CH=CH-	100	8	9	10	8
			150	9	12	11	10
			200	11	14	12	11
11	Standard	Ampicillin	10	20	19	17	19



Graph: I

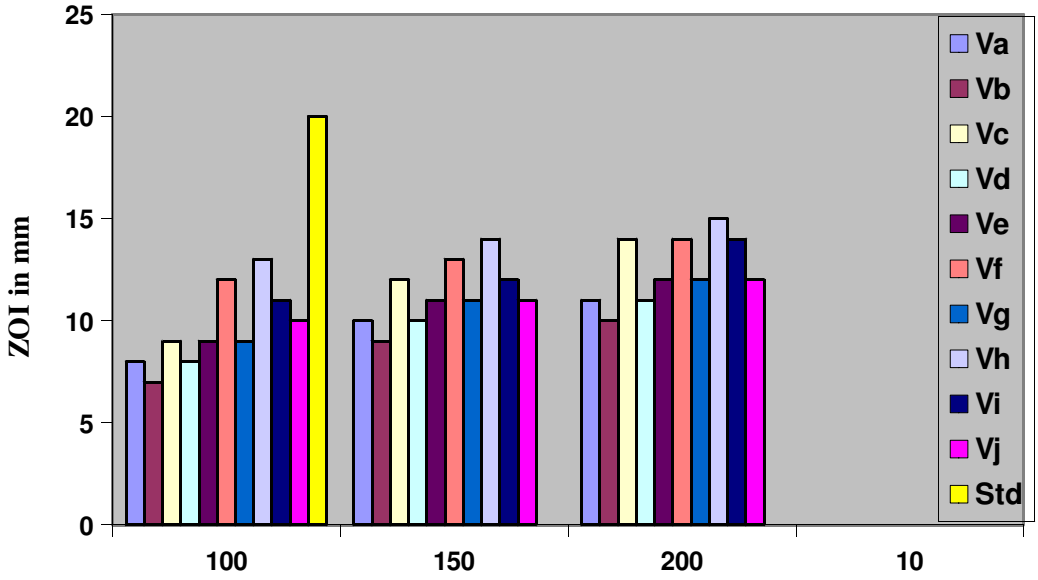


Graph: II



Graph: III

ZONE OF INHIBITION OF COMPOUNDS (Va - Vj) On E. Coli



Graph: IV

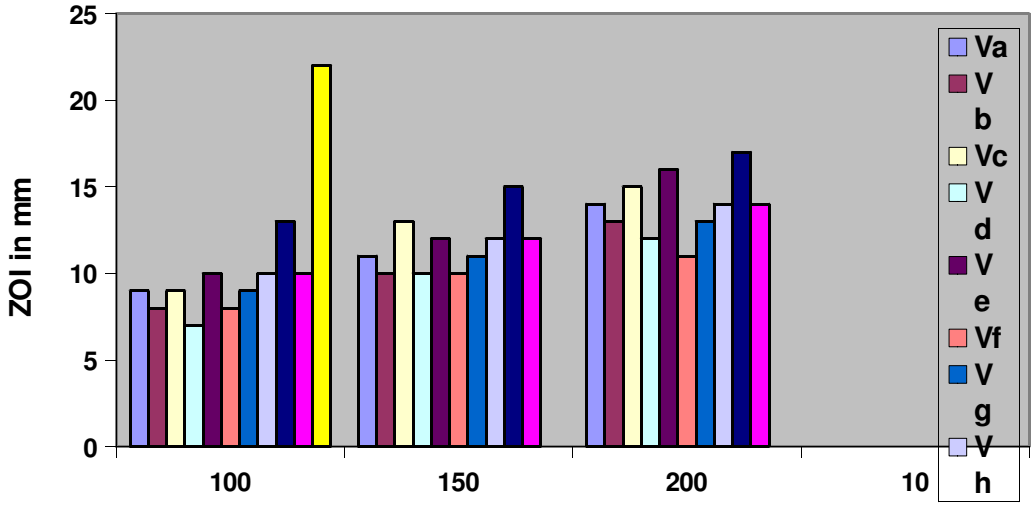
ANTIFUNGAL ACTIVITY

Table 5: Antifungal activity of 3-[(2)-(phenyl methylidene) hydrazono]-1, 3-dihydro-2H-benzo[*g*]indol-2-one derivatives.

S. No.	Substituents		Conc.	Zone of Inhibition (in mm)	
	Compounds	R	Conc. (µg/ml)	C. albicans	Yeast
1	Va	H	100	9	9
			150	11	10
			200	14	12
2	Vb	4-OCH_3	100	8	8
			150	10	9
			200	13	11
3	Vc	4-OH, 3-OCH_3	100	9	10
			150	13	11

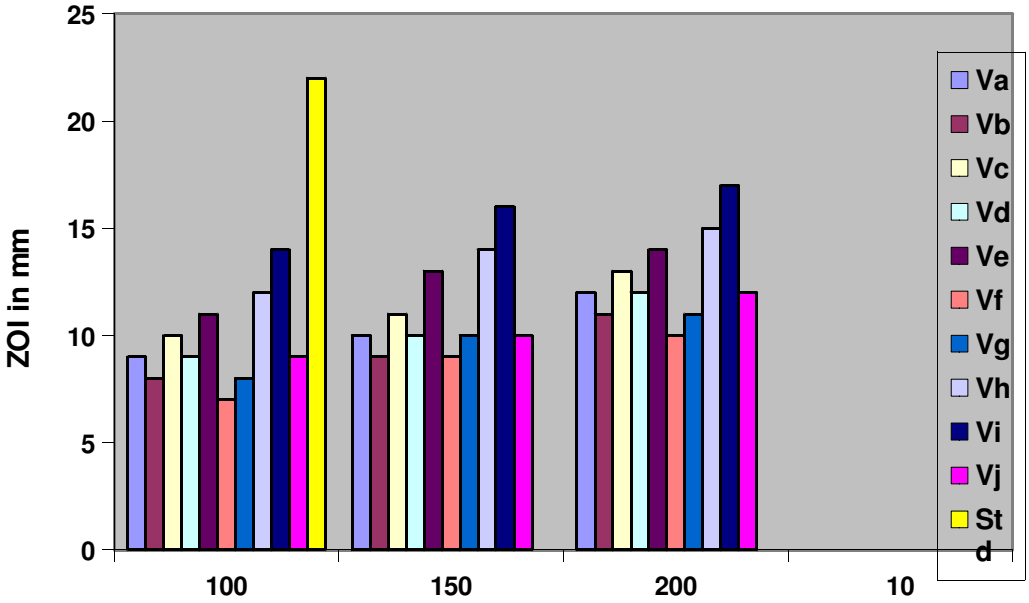
			200	15	13
4	Vd	4-Cl	100	7	9
			150	10	10
			200	12	12
5	Ve	4-NO_2	100	10	11
			150	12	13
			200	16	14
6	Vf	4-F	100	8	7
			150	10	9
			200	11	10
7	Vg	3-OCH_3, 4-OCH_3	100	9	8
			150	11	10
			200	13	11
8	Vh	2-OH	100	10	12
			150	12	14
			200	14	15
9	Vi	4-$N(CH_3)_2$	100	13	14
			150	15	16
			200	18	17
10	Vj	1-CH=CH-	100	10	9
			150	12	10
			200	14	12
11	Standard	Clotrimazole	10	20	19

ZONE OF INHIBITION OF COMPOUNDS (Va - Vj) On C.albicans



Graph: V

ZONE OF INHIBITION OF COMPOUNDS (Va - Vj) On YEAST



Conc. in µg/ml

Graph: VI

6. RESULTS AND DISCUSSION

Isatins (1H indole-2, 3- dione) are synthetically versatile substrates, where they can be used for synthesis of a large variety of heterocyclic compounds, Such as indoles & quinolines, and as raw material for drug synthesis. Isatin have also been found in mammalian tissue and their function as a modulator of biochemical processes has been the subject of several discussions.

The survey of the literature revealed that, isatin is a versatile lead molecule for designing potential bioactive agents, and its derivatives were reported to possess broad-spectrum antimicrobial, antiviral, anticancer, anti-inflammatory, anxiety, analgesic, anticonvulsant, antipsychotic activities. In the present study, we have investigated the 10 new isatin derivatives (Va-Vj), and tested for their antimicrobial activity.

Isatin (1H-indole- 2, 3-dione) was prepared by using Sandmeyer methodology; the title compounds (Va-Vj) were prepared from isatin, by using the reaction sequence in Scheme 17.

The newly Synthesized Isatin derivatives were purified by crystallization and chromatographic techniques (Thin Layer Chromatography). The chemical structures of synthesized compounds were confirmed by physico-chemical and spectral datas (IR, Mass and ^{1}H NMR studies).

All the synthesized compounds were tested for *in vitro* antibacterial activity by the agar diffusion method. The zone of inhibition values of the synthesized compounds against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* were presented in Table 4. Ampicillin was used for the reference for inhibitory activity against bacteria. It has been observed that all the tested compounds were showed mild to moderate activity against the bacteria, compound Vf (Fluro) was more potent anti- bacterial agent against both gram (+Ve) and gram (-Ve) organisms among all the test compounds. This was followed by compound Vi (N-dimethyl), Ve (Nitro) and Vh (Hydroxy).

The antifungal activities of the compounds were studied against *Candida albicans* and *Yeast.* Clotrimazole was used for the reference for inhibitory activity against fungi. All the tested compounds showed mild to moderate antifungal activity. Compound Vi (N-dimethyl) was more potent anti fungal agent among all the test compounds. This was followed by the compounds Vh (Hydroxy), Ve (Nitro) and Vc (4-Hydroxy-3-methoxy).

It has been felt necessary from the results of the present antimicrobial investigations that there is a need for further advanced studies, at least on the few of the test compounds which are found to be superior.

Ravi raj A. Kusanur *et al.,* [44] reported the synthesis of 4'-(coumarin-3-yl) spiro [3H-indol-3, 2'-1, 5-benzodiazepine]-2(1H)-one and evaluated for *in vitro* antibacterial activity. Among all the compounds, compound with R = 8-OCH_3, R^1 = R^2 = CH_3 showed 88.83% of inhibition against *B. subtilis* and 77.77% of inhibition against *E. coli* as compared to the standard and other compounds were moderately active.

Ankur patel *et al.* [87] reported the Synthesis and antimicrobial activity of some new isatin derivatives, substitution of chloro, bromo or fluoro groups produced more antimicrobial activity. Vijey Anandhi M *et al.,* [88] reported the Synthesis and antimicrobial activities of 1-(5-substituted-2-oxoindolin-3-ylidine)-4-(substituted pyridine-2-yl) thiosemicarbazide, showed the activity against Gram +Ve, Gram –Ve bacteria and fungi. The order of antibacterial activity of the substituents at the 5th position of isatin is Br > Cl > F.

From this investigation subtituents of fluoro, N-dimethyl, nitro and chloro groups containing compounds showed good antimicrobial activity.

7. CONCLUSION

Title compounds were showed mild to moderate activity against the bacteria.

In conclusion,

Vf (Fluoro) > Vi (N-dimethyl) > Ve (Nitro) > Vh (Hydroxy) > Vc (4-hydroxy-3-methoxy) > Vg (Dimethoxy) > Vj (Allyl) > Va (Hydro) > Vd (Chloro) > Vb (Methoxy).

Fluoro, N-Dimethyl, Nitro pharmacophore containing compounds shows significant lead optimization.

So further beneficial pharmacophore modifications in the design of isatin as promising anti-bacterial agent.

The title compounds were showed mild to moderate activity against fungi.

In conclusion,

Vi (N-dimethyl) > Vh (Hydroxy) > Ve (Nitro) > Vc (4-hydroxy-3-methoxy) > Vj (Allyl) > Va (Hydro) > Vg (Dimethoxy) > Vd (Chloro) > Vb (Methoxy) > Vf (fluro).

N-Dimethyl, Hydroxy, and Nitro pharmacophore containing compound shows significant lead optimization.

So further beneficial pharmacophore modifications in the design of isatin as promising anti-fungal agent.

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